FEASIBILITY STUDY FOR A SAFETY BACK-UP CRYOPRESERVATION FACILITY

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Cryobank at the International Institute of Tropical Agriculture
Credit: Shawn Landersz

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EXECUTIVE SUMMARY

This study was commissioned by Bioversity International, the International Potato Center (CIP) and the Global Crop Diversity Trust, and sponsored by the governments of Australia, Germany and Switzerland. The purpose of the study was to investigate the feasibility of establishing a safety back-up facility for cryopreserved collections of crops that are vegetatively propagated or have recalcitrant seeds. The study was undertaken by a group of external experts, supported by a task force of Bioversity, CIP and Crop Trust staff. The Expert Group drew on its knowledge of cryopreservation and biorepositories, crop conservation at national and international levels and genebank costing, as well as information and experience from Task Force members. The Expert Group investigated the state of crop cryopreservation. A survey was made of institutes with cryopreserved collections and information was collated on collections around the world held only in field and *in vitro* genebanks.

The study concludes that a major global initiative is urgently needed to accelerate the development and implementation of crop cryopreservation. The study highlights the advantages of cryopreservation for the long-term conservation of collections of vegetatively propagated and recalcitrant seed crops, but also the challenges faced with its wide-scale implementation. It recommends a collaborative effort among researchers and genebanks that is focused on the specific technical and practical issues hampering the adaptation of cryopreservation protocols to at-risk collections. The study findings indicate that 100,000 unique accessions of the vegetatively propagated and recalcitrant seed crops in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty) and in Article 15 collections are held in high maintenance, costly and potentially vulnerable field and *in vitro* genebanks.

The study recommends that a safety back-up cryopreservation facility is set up to accommodate the estimated 5,000 to 10,000 accessions arising from current, on-going cryopreservation activities at CGIAR and other genebanks. The importance of the facility being established and operated in accordance with best principles and practice is emphasized. The facility should follow the principles and policies that govern the Svalbard Global Seed Vault and its infrastructure and operations should adhere to established technical standards and practices for low temperature biorepositories.

CONTENTS

1. INTRODUCTION

Crop diversity is critical to global food security and sustainable agriculture. For many crops, their genetic diversity can be conserved *ex situ* for the long term in seed form. This is the case for crops such as rice and beans whose seeds tolerate desiccation (i.e. orthodox seeds) and can be dried, sealed in containers and stored in freezers or cold rooms. Most genebanks around the world have the equipment, trained personnel and funds to maintain seed crop collections in this way. However, there are crops whose diversity cannot be conserved for the long term as seeds. For example, banana has no seeds, coconut and cacao have seeds that do not tolerate desiccation (i.e. recalcitrant seeds), and crops such as potato and sweetpotato, apple and orange, are vegetatively propagated and do not breed true from seed. The diversity of these crops is typically maintained as living plants in field genebanks or as tissue cultures in *in vitro* genebanks. These methods have limitations for long-term conservation, and the cryopreservation of plant tissue, vegetative parts or embryos at -196 °C in liquid nitrogen offers a solution. Cryobanking is already very advanced for the storage of human, animal and microbial materials, but less so for plant conservation.

Many of the world's most important crops for food, nutrition and livelihoods, particularly for the poorest people, are vegetatively propagated or have recalcitrant seeds. This is reflected in the Plant Treaty which has the following vegetatively propagated and recalcitrant seed crops listed in Annex 1: apple, banana and plantain, breadfruit, cassava, *Citrus*, coconut, major aroids, potato, strawberry, sweetpotato and yams. In addition, international collections under Article 15 of the Plant Treaty include coffee, coconut and cacao, and a number of crops held by the CGIAR Centers (banana and plantain, potato, sweetpotato, cassava, aroids, yams and Andean roots and tubers as well as agroforestry species with recalcitrant seeds). Global annual production of vegetatively propagated crops and crops with recalcitrant seeds such as *Citrus*, banana/plantains, potato, sweetpotato and coffee is estimated to be more than one billion tonnes and worth at least US$100 billion annually (estimate based on FAOSTAT). More detailed information on some key vegetatively propagated and recalcitrant seed crops is presented in Appendix 1.

All crop diversity collections – whether held in national or international genebanks – face some risk of natural and human-made disasters, ranging from fire, flood and earthquakes to equipment failure, human error and war. Therefore, for security reasons, collections are typically duplicated in more than one genebank. In 2008, the Svalbard Global Seed Vault was established to provide an additional level of security for crop diversity collections with orthodox seeds. Built and owned by the government of Norway, the Seed Vault takes advantage of the naturally low temperature and remoteness of the arctic environment of Svalbard. It is available for use by countries, international institutes and other organisations, public and private. Collections are deposited under the terms of an agreement between the depositor and the Norwegian Ministry of Food and Agriculture wherein the rights of control over the deposited materials remain with the depositing institution: a so-called 'black box' agreement. The Seed Vault operates within the framework of the Plant Treaty and is managed by the Nordic Genetic Resource Centre, supported by the Global Crop Diversity Trust and overseen by an International Advisory Council.

There is no Svalbard Seed Vault equivalent for crops that are vegetatively propagated or have recalcitrant seeds, yet the use of cryopreservation as a long-term conservation method is on the increase. The CGIAR Centers – notably Bioversity International for *Musa* and the International Potato Center (CIP) for potato – have cryopreserved collections, and a number of other genebanks and institutions around the world are also engaged in cryobanking. For these reasons, Bioversity, CIP and the Crop Trust initiated this study on the feasibility of establishing a cryopreservation facility that can serve as an ultimate safety back-up for vegetatively propagated and recalcitrant seed crops.

2. STUDY PURPOSE AND PROCESS

The purpose of this study is to investigate the feasibility of establishing a safety back-up facility for cryopreserved collections of plant genetic resources for food and agriculture that are vegetatively propagated or have recalcitrant seeds.

Bioversity, CIP and the Crop Trust established an inter-agency Task Force to initiate and oversee the study by external experts. The Australian Centre for International Agricultural Research, the Swiss Agency for Development and Cooperation and the German Federal Ministry for Economic Cooperation and Development provided financial support. The Task Force, in consultation with the donors, developed the terms of reference for the study and engaged an Expert Group to undertake the study (Annex 1: Terms of Reference and members of the Expert Group and the Task Force).

The Expert Group and Task Force met online on 31 March 2017 to review the terms of reference and discuss a background document provided by the Task Force. They agreed on a work plan and *modus operandi*, with tasks distributed among the members of both groups. The work plan focused on assessing cryopreservation technology, the extent of its current and likely future use, and the technical, policy and cost requirements of a safety back-up cryopreservation facility.

The processes for addressing these issues are described below:

i. Cryopreservation as a long-term conservation method

   Information from published and grey literature and data from Bioversity and CIP were compiled to compare the practicality, reliability and cost-effectiveness of cryopreservation with field and *in vitro* methods, for maintaining the viability and genetic integrity of plant genetic resources collections over the long-term (>50 years). The state of development of cryopreservation protocols and the challenges of cryopreserving collections of vegetatively propagated and recalcitrant seed crops were also assessed.

ii. Assessment of current and future potential use of a safety back-up cryopreservation facility

   Twenty-six institutions were sent a questionnaire aimed at assessing their current scale of cryobanking and the likelihood of an increase over the next five years. The questionnaire also addressed duplication of cryopreserved collections and interest in a safety back-up cryopreservation facility. The surveyed institutions were known to have cryopreserved collections or thought likely to engage in cryobanking in the near term. They included the five CGIAR genebanks that hold vegetatively propagated or recalcitrant seed crops (Bioversity International, International Center for Tropical Agriculture (CIAT), International Potato Center (CIP), International Institute of Tropical Agriculture (IITA), World Agroforestry Centre (ICRAF)), as well as national genebanks and universities. The questionnaire, introductory letter and list of surveyed institutes can be found in Annex 2. The responses were compiled to provide an estimation of the potential demand for a safety back-up cryopreservation facility in the near term.

   In addition, an assessment was made of the collections of vegetatively propagated and recalcitrant seed crops that are in field and *in vitro* in genebanks around the

world and not yet the object of cryopreservation. This provides an indication of the potential future use of a safety back-up cryopreservation facility should these collections eventually be put into cryopreservation. The assessment was based on information from the Food and Agriculture Organization of the United Nations (FAO) State of the World Report on Plant Genetic Resources for Food and Agriculture, FAO World Information and Early Warning System (WIEWS), the genebank online information portal Genesys, Crop Trust project documents and global crop conservation strategies and the websites of individual plant genetic resources institutes (Annex 6). The data gathering focused on the vegetatively propagated and recalcitrant seed crops and collections included in Annex 1 and Article 15 of the Plant Treaty.

iii. Policy and technical requirements for the location and operation of a safety back-up cryopreservation facility

The principles and policies governing the operations of the Svalbard Global Seed Vault were reviewed for their relevance to a safety back-up cryopreservation facility. Information on cryobanking in other fields, in particular for medical repositories, and CGIAR experience were drawn on for assessing design and infrastructure requirements. A variety of sources were consulted with regard to technical standards, including the International Society for Biological and Environment Repositories (ISBER), the FAO Genebank Standards and the Standard Operating Procedures (SOPs) used by the CGIAR genebanks. The documentation consulted is listed in Annex 6.

iv. Costs of establishing and running a safety back-up cryopreservation facility

CGIAR Centers and commercial biobanking sources were drawn on to estimate the costs of storing cryopreserved materials and to set-up and run a cryopreservation facility. These initial estimations were made based on a number of assumptions described in detail in the study.

The Expert Group and Task Force reviewed progress on information gathering and background studies at periodic on-line meetings and discussed the findings at the Crop Trust in Bonn on 15 and 16 May 2017. Participants also agreed on timing and responsibilities for drafting this report. The agenda of the Bonn meeting can be found in Annex 3.

The results of the analysis into the state of cryopreservation and the Expert Group's conclusions and recommendations are presented below in Sections 3 and 4, respectively.

The draft report was circulated to the institutes with cryopreserved collections that responded to the survey. Their compiled comments are in Section 5.

3. ANALYSIS OF THE STATE OF CONSERVATION OF VEGETATIVELY PROPAGATED AND RECALCITRANT SEED SPECIES

3.1 Cryopreservation as a long-term conservation method: the pros and cons

Cryopreservation is the process of preserving the biologic structure and/or function of living systems by freezing to, and storage at, ultra low temperatures. Cryopreservation uses the effect of decreased temperature to suppress molecular motion and arrest metabolic and biochemical reactions. Below -150 °C, a state of "suspended animation" can be achieved in samples that are appropriately protected from the damage that occurs during freezing and thawing and there are very few biologically significant reactions or changes to the physicochemical properties of the system that occur below this temperature (Mazur 1964). Cryopreservation is the only current technology that provides safe long-term conservation of biological material as it maintains *ex vivo* biologic function, does not induce genetic alterations (Harding 2004) and provides long-term stable storage.

Evidence for the longevity and genetic stability conferred on plant samples by cryopreservation was found in a survey of the research literature, as presented in Appendix 2. This contrasts with the experimental evidence of somaclonal variation of tissue conserved as *in vitro* cultures and the risks to plant survival in the field.

A survey of the literature on the costs of conservation has shown that, depending on the crop and type of material, introducing an accession into cryopreserved storage is more expensive than establishing an accession in *in vitro* culture or in the field. However, the costs of maintaining an accession in cryopreserved storage for the long-term (over 20 years) are considerably lower than those of maintenance in the field or *in vitro*, particularly when dealing with a large number of accessions. The data and references on these comparative costs are given in Appendix 2.

In summary, the 'pros' of cryopreservation are the low costs, greater longevity and high degree of genetic stability in maintaining collections for the long-term compared to other conservation methods. The 'cons' are the high cost of putting collections into cryopreservation in the first instance.

3.2 State of conservation of vegetatively propagated and recalcitrant seed crops

A survey was conducted of twenty-six institutes comprising international and national genebanks in developed and developing countries, including five CGIAR Centers, as well as universities and other research organisations. These 26 institutes were identified as potential users of a safety back-up cryopreservation facility in the near term because they were known to be cryopreserving collections of vegetatively propagated and/or recalcitrant seed crops, or thought likely to begin to do so within the next few years. Nineteen institutes responded with information

and their responses are presented and discussed in Appendix 3. Of these, 15 have cryopreserved collections. The key findings below are drawn from the information they provided. Two major institutes for cryopreservation, one in the United States of America (USA) and one in the Republic of Korea, did not provide information. They are known to hold large collections of apple and *Allium*, respectively.

Below are the key findings from 15 institutes (full data in Appendix 3, Table 3-1).

3.2.1 The number and size of collections in cryopreservation are small in comparison to collections conserved in the field or *in vitro*

The 15 institutes together hold 9,650 accessions of 30 crops[1] in cryopreservation. This constitutes only 16% of the total number of accessions they collectively hold of these crops. The majority of the accessions are maintained in the field (66%) and/or *in vitro* culture (46%), as shown in Figure 1.

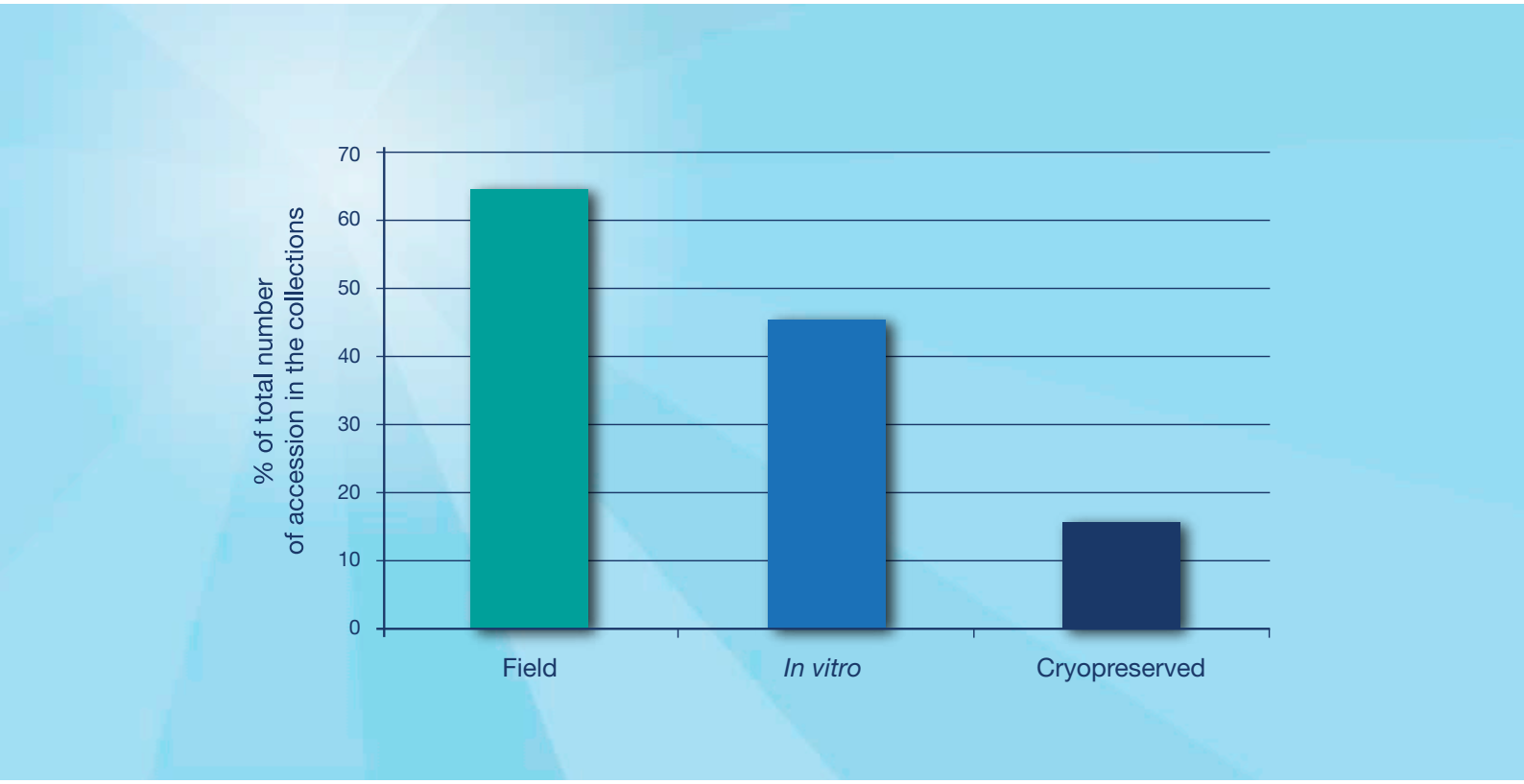


Figure 1. Percentage of the total holdings of 15 institutes for 30 crops that are maintained in cryopreservation, in *in vitro* culture and in the field.

The small number and size of cryopreserved collections is the likely consequence of the technical and financial challenges faced in implementing cryopreservation (Section 3.3 below). Because of these challenges, cryopreservation is typically directed at securing the unique and distinct accessions, therefore the cryopreserved collection can be expected to be of smaller size than the total collection.

[1] The "crop" is defined as all species and cultivars within the same genus, e.g. "banana and plantain" includes all species belonging to *Musa*.

3.2.2 The range of crops represented in cryopreserved collections is limited

Only potato, mulberry, strawberry, banana and plantain, *Allium*, cassava, coffee and mint have cryopreserved collections of more than 100 accessions, as shown in Figure 2 (full data in Appendix 3, Table 3-1). (Note: this does not include collections from institutes not participating in the survey such as the apple cryopreserved collections in the US).

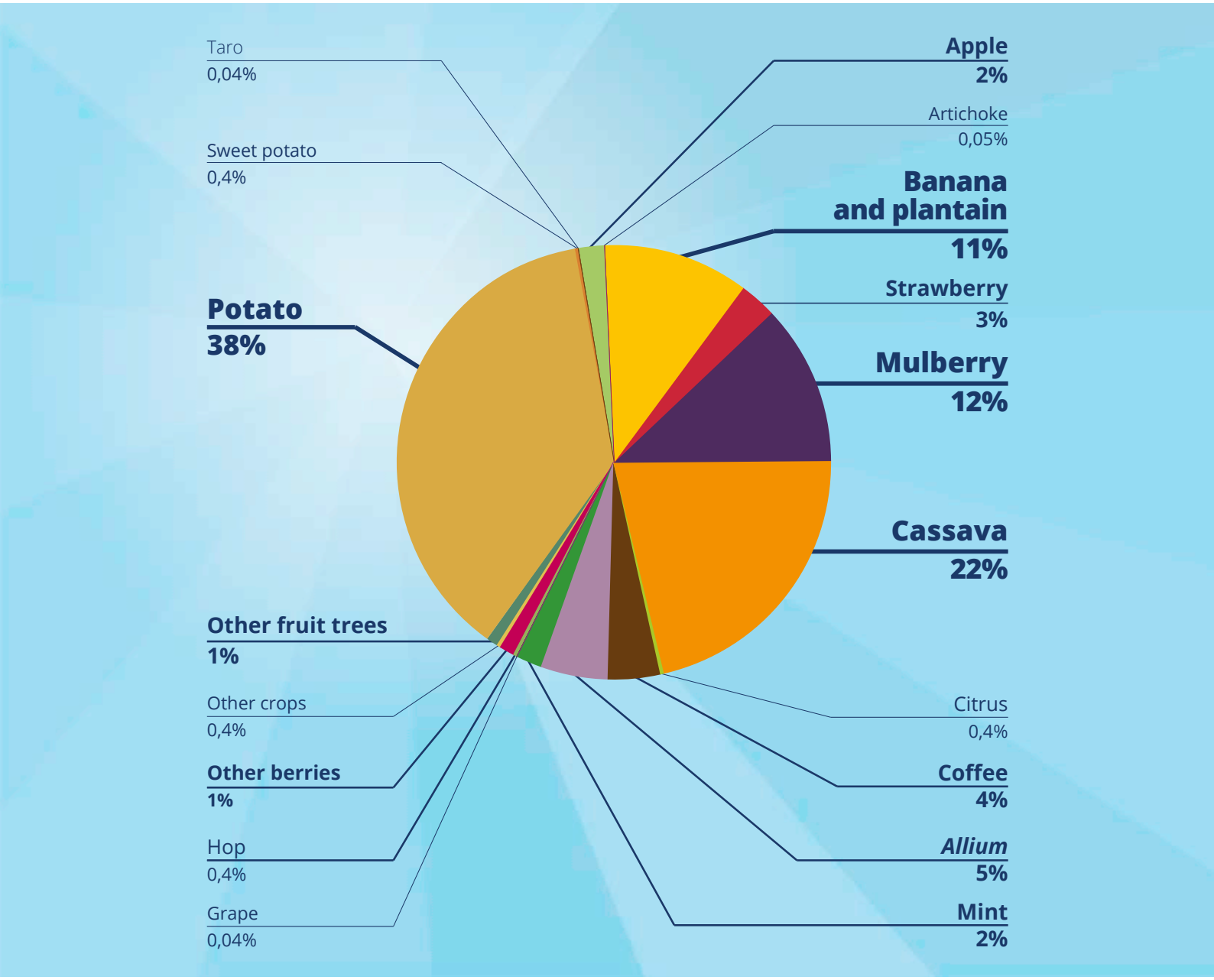


Figure 2. Representation of different crops in the cryopreserved collections of the 15 institutes.

These eight crops have well-developed protocols that have been successfully implemented across a wide range of diverse accessions. However, not all institutes experience the same success in implementing a protocol. The issues in developing and implementing cryopreservation protocols are discussed in Section 3.3, below.

3.2.3 For the majority of crop collections held by the 15 institutes, less than 40% is cryopreserved

The only exception is banana and plantain. With 66% of the banana collection at Bioversity cryopreserved, the average across all the banana and plantain collections is over 50% as shown in Figure 3.

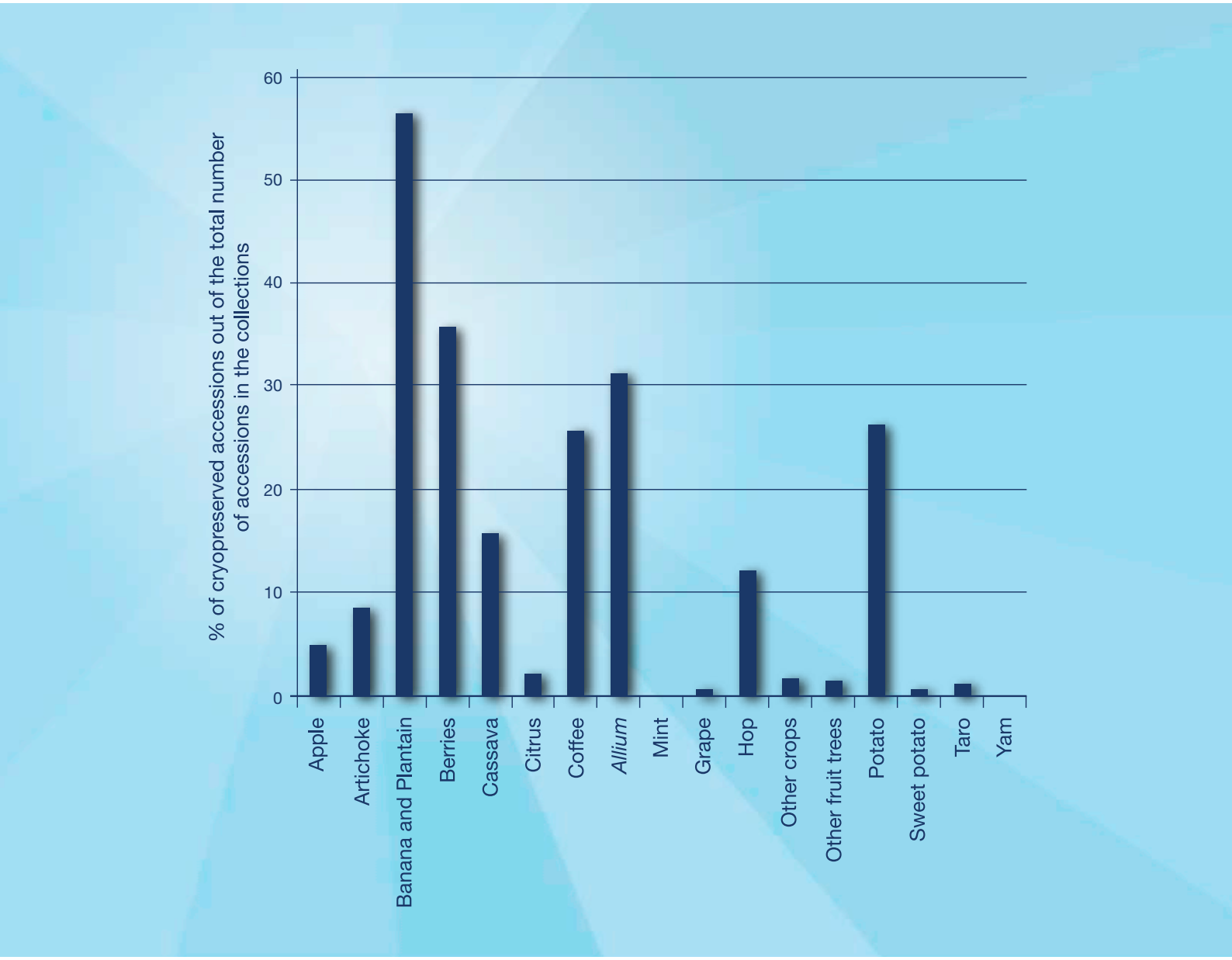


Figure 3. Proportion of accessions in cryopreservation out of the total number of accessions held collectively by the 15 institutes. Note: "Berries" include *Morus* spp. (mulberry), *Fragaria* spp. (strawberry), *Rubus* spp., *Ribes* spp., and *Lonicera caerule* (based on data in Appendix 3, Table 3-1).

3.2.4 The scale of cryopreservation varies significantly among the 15 institutes

This difference is illustrated in Figure 4 using apple and potato as examples. In institutes, with small collections, 100% of a collection may already be cryopreserved. Where collections are large, the percentage of the total collection in cryopreservation is less than 50%, and in some cases very small. This shows that even for crops with fully operational protocols such as potato and apple, there is still much work to do to fully cryopreserve collections. This is a reflection of the complexities and time involved in cryopreservation that are discussed in Section 3.3 and Appendix 5.

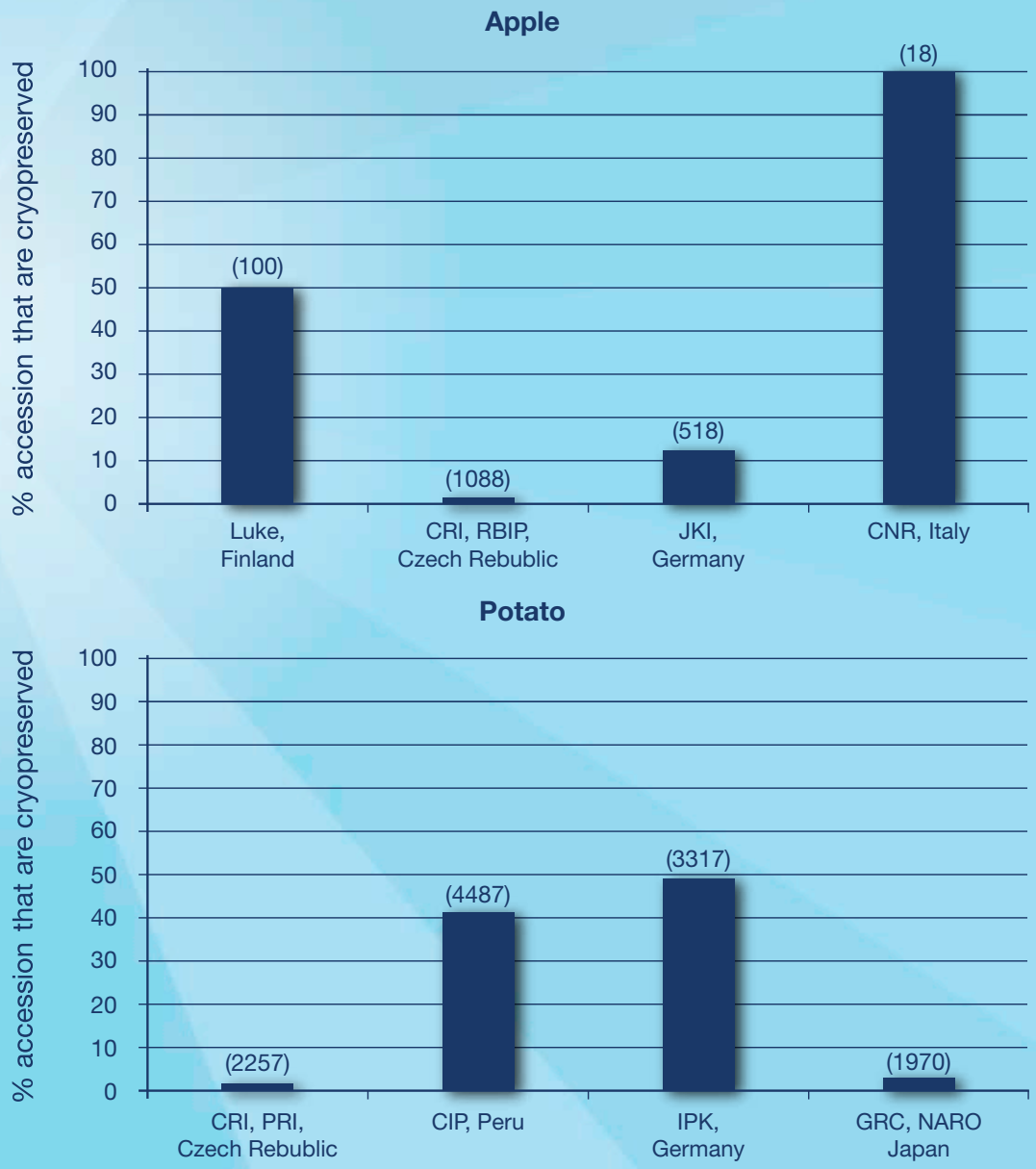


Figure 4. Number of accessions in cryopreservation as a proportion of the total number of accessions in the apple and potato collections of some of the institutes surveyed. Numbers in brackets indicate the total number of accessions held.

3.2.5 The capacity requirement for a safety back-up cryopreservation facility is approximately 5,000 accessions rising to 10,000 accessions over the next five years

Currently, the 15 institutes together hold about 9,650 cryopreserved accessions and this is projected to increase to 15,526 by 2022 (full data in Appendix 3, Tables 3-1 and 3-2). However, only eight of the institutes indicated that they would have sufficient number of cryopreserved samples to be able to make a deposit to the back-up facility should it become available in 2018. Together, these eight institutes expect to be able to deposit 6,217 accessions in 2018 and 11,412 by 2022 (full data in Appendix 3, Table 3-4). This represents a relatively limited need for a safety back-up cryopreservation facility at present and over the coming five years, unless there is a substantial increase in the scale and rate of cryopreservation.

In addition to the survey of institutes involved in cryobanking, information was gathered on collections of Annex 1 crops and Article 15 collections that are vegetatively propagated or have recalcitrant seeds that are in field and *in vitro* in collections around the world and not yet the object of cryopreservation activities. This was undertaken to provide an indication of the potential future use of a safety back-up cryopreservation facility should these collections eventually be cryopreserved. The results of the data gathering show that there is a total of approximately 400,000 accessions in mainly national genebanks, that are held only in the field or in *in vitro* culture (Appendix 4, Table 4-1). There is likely to be significant duplication among these collections. Even assuming that only one in four accessions is distinct, there could be as many as 100,000 distinct accessions not yet cryopreserved and secured for the long-term. This gives an indication of the potential future use of the safety back-up facility when the scale and rate of cryopreservation activity grows.

Given the constraints in budget, infrastructure and personnel that many genebanks face, especially those of National Agricultural Research Institutes (NARIs) in developing countries, it is unlikely that these 100,000 accessions can be secured in cryopreservation without substantive support and capacity building. For some of the crops concerned, cryopreservation protocols have been developed. In some cases, they are in routine use by other genebanks; in other cases, they have been developed but are not routinely applied because they require adaptation to the specific material and working environment in question. Only with a major initiative on cryopreservation, providing direct support and capacity-building, will many NARIs be able to make use of a safety back-up cryopreservation facility to secure the collections they hold.

3.3 Challenges in implementing plant cryopreservation

The institutes that were surveyed were asked to list the major issues they face in implementing cryopreservation. Their responses are summarized in Figure 5 below.

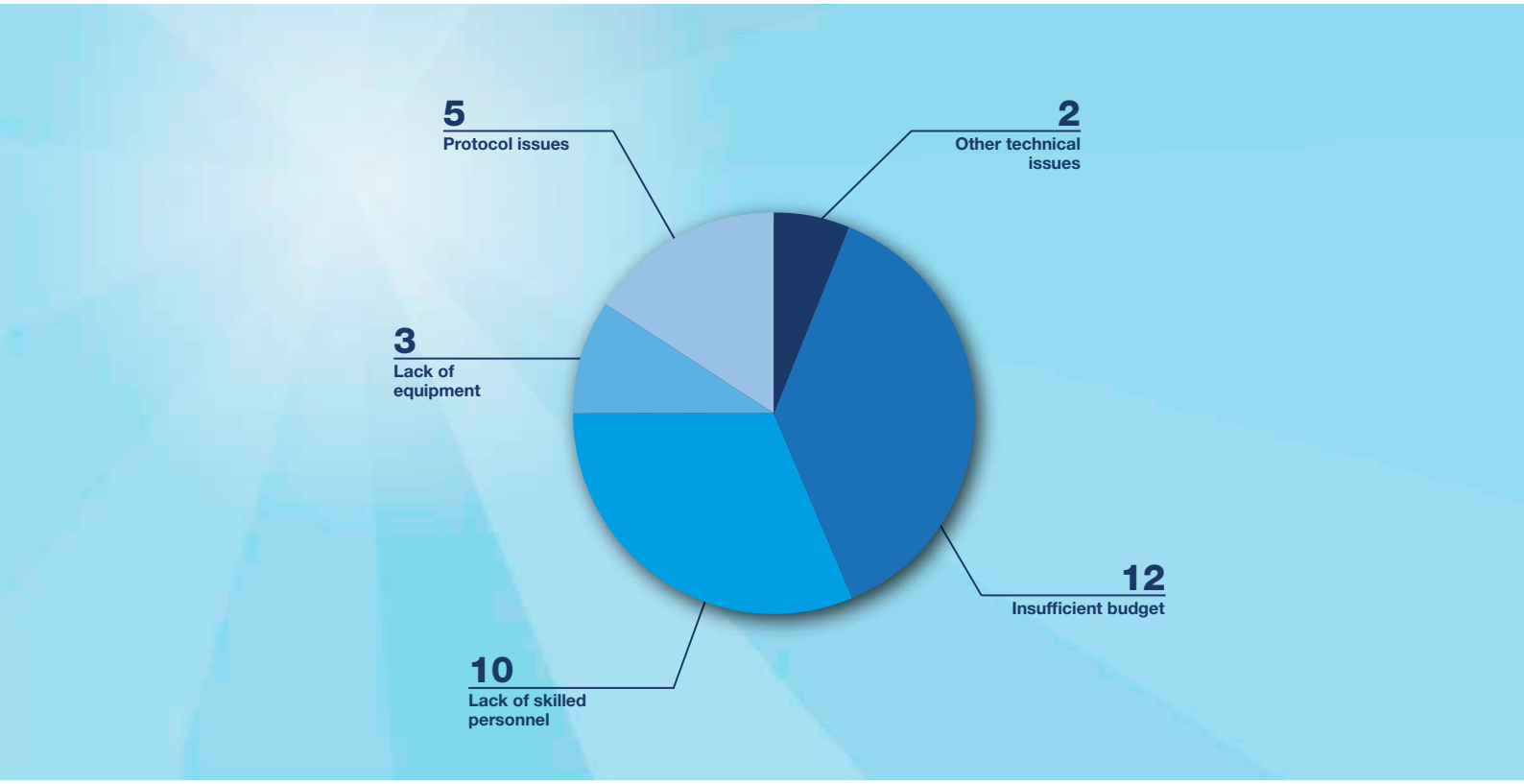


Figure 5. Major challenges in implementing the cryopreservation of collections as reported by the 19 institutes that responded to the survey (numbers on the chart represent the number of institutes that reported the specific constraint).

These challenges hamper the implementation of cryopreservation despite its significant advantages for long-term conservation over more traditional conservation methods (field and *in vitro*). Cryopreservation is currently utilized at a large scale by only a few genebanks and laboratories in the world. The challenges can be categorized as follows:

i. Challenges in protocol development (the science and methodology of cryopreservation)

ii. Challenges with the implementation of existing cryopreservation protocols (the genotype-specific issues in adapting the protocols to multiple accessions; effective work organization; sufficient supply of plant material, etc.)

iii. Challenges related to cryobanking capacity (insufficient funding, lack of skilled personnel, lack of equipment/infrastructure, etc.)

Although funding is shown as a constraint by 50% of the genebanks surveyed, a fundamental issue in plant cryopreservation is the lack of repeatability of protocols from one laboratory to another, and the difficulty in applying experimental protocols on the wide range of diversity found in genebanks. These issues are reflected in the analysis of responses to the question in the survey where the institutes were invited

to list the crops for which cryopreservation is not routinely implemented (Appendix 3, Table 3-2). Some of the crops listed, such as garlic, potato or sugar cane, have already been successfully cryopreserved on a relatively large scale in other institutes. The difficulty in adapting the existing protocols (lack of reproducibility) can be due to numerous causes, ranging from different sources of laboratory supplies to the different equipment and the different levels of technical skills found in different cryopreservation laboratories. This latter factor, the availability of skilled personnel, was one of the top limitations mentioned in the survey. However, all too often, even with the highest skilled cryopreservation personnel, there is a finesse point in applying cryopreservation protocols in plants from one laboratory to another that cannot be easily explained. This adds to the difficulty in establishing quality management programmes that are 100% applicable across cryobanks and in doing any reciprocal testing of protocols.

The regeneration of whole plants from cryopreserved small organs (shoot tips or embryos) is another limitation faced in cryopreservation. Successful plant regeneration requires not only the cryopreservation protocol to be optimized but also the post-cryopreservation conditions (e.g. *in vitro* culture conditions).

In short, modern cryopreservation protocols are complex, multi-stage processes that should be implemented by skillful personnel and require additional adjustment when transferred between genebanks and applied to diverse accessions. Plant cryopreservation scientists have a long way to go to understand all the variability inherent in this system and this, plus inadequate funding, helps explain the relatively few big plant low temperature biorepositories when compared to animals. Last but not least, because of the technical and cost constraints, many genebank managers still consider cryopreservation an area of research, with no direct application for the safeguard of their collections, despite the potential advantages of the technology for long-term conservation.

The challenges are discussed in more detail in the Appendix 5.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Need for a safety back-up cryopreservation facility

There are cryopreserved collections of vegetatively propagated and recalcitrant seed crops that are not duplicated for safety. The survey of institutes holding cryopreserved collections revealed that only five have parts of their collections safety duplicated elsewhere which amounts to only about a third of the total holding of cryopreserved accessions (Appendix 3, Table 3-3).

Some of the institutes responding to the survey expressed interest in using a safety back-up cryopreservation facility, but not all (Section 3.2; Appendix 3, Table 3-4). Significantly, only eight institutes responded that they would have sufficient replicate samples of their accessions in cryopreservation to be able to send a portion (e.g. two or more replicates) for deposit into a back-up facility. Based on the projections of these eight institutes regarding their deposits, it is estimated that the backup cryopreservation storage facility would need an initial capacity of approximately 5,000 accessions increasing to 10,000 by 2022. These estimates include the safety back-up requirement for the international collections of vegetatively propagated and recalcitrant seed crops held by the CGIAR Centers.

The current need, therefore, is for a small-scale facility to host safety back-ups of existing unique cryopreserved accessions. Over time, the facility will need to increase capacity in line with on-going efforts to cryopreserve collections. However, given the limited number of institutes currently engaged in cryobanking and likely to use the facility, only modest increases in capacity are foreseen for the near term, perhaps 1,000 accessions per year.

With the current low level of cryobanking and the challenges hampering progress (as outlined in Section 3.3), it is likely to be a decade or more before a back-up cryofacility could reach the scale of what might be considered a global safety repository for all vegetatively propagated and recalcitrant seed crops of importance for world food security and livelihoods. At present, only six of the 13 crops on Annex 1 or in Article 15 collections that are vegetatively propagated or have recalcitrant seeds are known to have collections in cryopreservation. This slow progress in the development and implementation of plant cryopreservation also constrains the CGIAR genebanks in meeting their obligations to safeguard the international collections of crops that are vegetatively propagated or have recalcitrant seeds under their care.

At this time, therefore, the most pressing need is for a global initiative to accelerate the development and implementation of cryopreservation of vegetatively propagated and recalcitrant seed crops. In addition to the backlog in current crop cryopreservation efforts, there are an estimated 100,000 unique and distinct accessions held only in the field and *in vitro* culture, and thus at risk of loss, especially to pests and diseases (Section 3.2). Safeguarding these valuable but vulnerable accessions, many of which are in national genebanks, will only be possible with

a major programme of capacity-building and wide-scale implementation of cryopreservation. This is discussed further under Section 4.2 below.

However, as pointed out above, there are some 5,000 accessions rising to 10,000 that are already in need of safety back-up, or will be in the near term. This includes the international collections held by the CGIAR Centers, where about 3,000 cryopreserved accessions are ready for safety back-up. Therefore, it is proposed that the CGIAR genebanks take the lead to organise the setting-up of a safety back-up cryofacility that they can use to secure the international collections, but that is also available for use by other genebanks, in particular those that said in the survey that they would be willing to use such a facility.

One 800 L cryostorage container will accommodate 10,000 accessions. This equipment requirement is not excessive; many cryobiology laboratories will have equipment of this type and capacity. However, a cryofacility that is expected to operate for the long term, and provide a safety back-up service available to genebanks around the world, must necessarily meet stringent technical as well as policy requirements. Small or large in size, the safety back-up cryopreservation facility needs to follow best principles and practice for its operation. These aspects are discussed in Sections 4.3 and 4.4 below.

The facility will grow in capacity over time as accessions are cryopreserved and deposited. However, as pointed out earlier, unless there is a step change in the development and implementation of plant cryopreservation, there will be no compelling need for a large global safety back-up facility. The CGIAR Centers are also well placed to lead the process to develop the plans and secure the funding for a major initiative to accelerate cryopreservation (Section 4.2).

The challenges faced in cryobanking (Section 3.3) may mean that some prospective depositors to the safety backup facility, may choose not to invest in the specialized infrastructure needed to manage cryopreserved collections over the long-term in their own institute. In these cases, the facility would be the first-level back-up to collections held only in the field or *in vitro* by the depositing genebank. With no duplicate sample under cryopreservation, and given the vulnerability of field and *in vitro* collections, depositors may need to access the facility more frequently. However, it is foreseen that this can be accommodated in a technically sound and cost-effective manner.

4.2 Advancing the cryopreservation of crop collections

The cryopreservation of crop collections is still in its infancy. The analyses in Section 3 show that only a few genebanks (ca. 15) routinely cryopreserve crop collections and relatively few crops have fully operational protocols, i.e. tested for >50 accessions (i.e. potato, apple, banana and plantain, cassava, *Citrus*, berries, garlic, coffee). Furthermore, on average only 40% of the collections are secured in cryopreservation, with another 100,000 unique accessions, not yet the target of cryopreservation efforts, remaining at risk in the field and *in vitro* culture (Section 3.2.). To safeguard the diversity of vegetatively propagated and recalcitrant seed crops, cryopreservation needs to be greatly accelerated.

A global initiative is needed on the development and implementation of cryopreservation, for two main reasons. Many important crops, in particular those critical for the food security and livelihood needs of the world's poorest, are vegetatively propagated or have recalcitrant seeds (see Appendix 1). Cryobanking is more cost-effective for long-term conservation than *in vitro* tissue culture or field genebanks, offering a cheaper option and better guarantee for the maintenance of viability and genetic stability over time (Section 3.1).

Such an initiative requires a coordinated effort to expedite the development of the global expertise in the cryopreservation of vegetatively propagated and recalcitrant seed crops. A coalition of genebanks, researchers, advocacy organizations, academic institutions, and other stakeholders should be assembled to address the unmet need for cryopreservation advances, outlining remaining challenges and identifying areas of underinvestment and untapped opportunities. The initiative would need to address the challenges in protocol development and their adaptation to a broad range of diversity in different crop collections as described in Section 3.3 and Appendix 3. However, if cryopreservation is to be accelerated, the initiative must include a coordinated programme of support and capacity building to implement and enhance the cryobanking of priority collections. Priority should be given to the Article 15 international collections and the unique and distinct collections of Annex 1 crops in genebanks around the world that are vegetatively propagated or have recalcitrant seeds. Examples of what is needed for a selection of high priority crops are given in Appendix 6. Advancing the development and implementation of cryopreservation would give purpose and impetus to a safety back-up cryopreservation facility in assuming a global service role.

However, there will be prospective depositors to a safety back-up cryopreservation facility that do not have the capacity to undertake the cryopreservation of their collections themselves. This could be addressed through the initiative and/or by service provision whereby a third party undertakes the cryopreservation under contract to the depositor. Given the species-specificity of cryopreservation protocols and the various quarantine regulations on the safe movement of seeds, tissues and vegetative parts, such service provision contracts are best carried out by institutes with relevant crop-specific expertise and could themselves include a training

component for the depositing institute. Such service providers would be partners in the initiative to further the development and implementation of cryopreservation.

As mentioned in Section 4.1 above, it is proposed that the CGIAR Centers initiate a process to develop the plans and secure the funding for a global initiative to further the development and implementation of crop cryopreservation. With priority on the Article 15 collections and Annex 1 crops of the Plant Treaty, such an initiative will support the Centers and other genebanks in cryopreserving important vegetatively propagated and recalcitrant seed crops and give greater momentum and purpose to the development of a safety back-up cryopreservation facility.

4.3 Principles and policies for the operation of a safety back-up facility

It is recommended that the back-up cryopreservation facility, in whatever form it takes, operate according to the same principles and policies that govern the Svalbard Global Seed Vault. Experience shows that these principles and policies have satisfactorily met both the political and practical expectations of the world community. Importantly, they will establish the facility as providing an international service, as opposed to simply meeting strictly national, institutional or even individual needs.

The cryopreservation facility will be responsible for the long-term safety back-up storage of cryopreserved collections of plant genetic resources that are vegetatively propagated or have recalcitrant seeds, with priority given to the international collections under Article 15 of the Plant Treaty and national collections of crops listed on Annex 1.

Rights of ownership and control over the deposited materials will remain with the depositing institution under a black-box agreement. The use of the facility will be voluntary and retrieval of deposits will be 'as a last resort,' i.e. only when the accessions are no longer available or at dangerously low viability in the depositor's or another genebank. The facility will operate within the framework of the Plant Treaty.

It is proposed that the facility be hosted in a country that has ratified the Plant Treaty and that its operations are overseen by an international advisory council comprising representatives from the Plant Treaty, FAO, Crop Trust, CGIAR, facility host, manager and other appropriate bodies. The eligibility of collections for deposit in the facility would be determined by the advisory council. The principles, policies and responsibilities would be laid out in an agreement between the host and depositor. A technical committee will be needed to ensure that the cryopreservation facility's infrastructure meets international standards and that depositors are provided with guidelines on best practice for preparing, packaging and shipping collections to the facility.

The management of deposits would be best vested with an institute engaged in plant genetic resources conservation. However, the actual location and day-to-day maintenance of the storage facility could be assigned or contracted out to an institution that has a strong experience with cryobanking and biorepository management, in or outside of the plant genetic resources field. Options on locations are discussed in Section 4.5, below.

4.3.1 Host obligations

The host institution will be responsible for ensuring that the cryopreservation facility has political backing and secure funding over the long-term. Both are essential for the sustainability of the facility. The host institution will need to provide the legal and administrative functions of the facility, including issuance of the depositor agreement and any other arrangements needed for maintenance, management and/or funding of the facility. The host will need to obtain a waiver for phytosanitary inspections from the host country's plant quarantine authority. It will also need to organize any necessary import and phytosanitary certification processes.

The host institution will ensure that the cryopreservation facility is protected from natural and human-made hazards to the extent possible. It will ensure the establishment, maintenance and operations of the facility, providing equipment, supplies and monitoring and back-up systems as well as the technical and administrative personnel needed to run the facility. Personnel may be provided by the host itself, or through arrangement with other appropriate organizations. The host institution will also be responsible for putting into place the necessary arrangements for receiving, storing and repatriating deposits in accordance with the deposit agreements and agreed technical standards. These actions may be undertaken by the host itself, or by arrangement with an appropriate technical institute. The duties would include the administration of the deposit agreements and also import/export and phytosanitary certification. They would involve maintaining a publicly available register (database) of the deposits and making the facility's monitoring records available, as well as promoting the role of the facility. The work and meetings of the facility's international advisory council and technical committee would need to be supported.

4.3.2 Depositor obligations

The depositor must disclose the content of the deposit so that its eligibility can be ascertained (i.e. provide the passport data). The depositor's institute must agree to make the accessions it maintains available to users under the Treaty's Multilateral System or on equivalent terms. The depositor is ultimately responsible for the health and viability of the deposited material. To help ensure that the deposit is of adequate quality and appropriately documented, the cryopreservation facility will require adherence to the following technical guidelines:

- Material conforms to recommended standards for acceptable viability and health status, number of replicates, etc.
- Required passport data are provided for each accession, to be included in the facility's publically available registry of deposits.
- Electronic and paper documentation are provided on the cryopreservation protocol and method for plant regeneration, to be archived at the facility.
- Required phytosanitary, export and import certificates are provided.
- Samples are packed and labeled according to recommendations and shipped to the facility in a dry shipper that will be provided as needed by the cryopreservation facility.

4.4 Infrastructure and technical requirements for the facility and its operations

Cryopreservation requires specialised high-tech equipment, monitoring and back-up systems. Based on current demand for a safety back-up system to accommodate the current 5,000 accessions, which could grow to 10,000 accessions over the next five years, a cryopreservation storage facility would require one primary 800 L storage container with a single back-up container. As the number of accessions grows with the increase in activities proposed through a global initiative on plant cryopreservation, the number of storage containers will need to expand to 8-12 storage containers to accommodate the estimated need for an additional 100,000 accessions.

The infrastructure needs to be established and run according to best available standards of practice to ensure the necessary quality and safety of its operations. Manipulating liquid nitrogen and maintaining ultra-low temperature storage tanks at -196 °C is not without serious hazard for the material being stored as well as for the technicians operating the tanks. Cryobanking is well developed for repositories of biological materials, particularly in human and veterinary medicine and assisted reproduction. The International Society for Biological and Environmental Repositories (ISBER) would be a major resource for determining best practices in infrastructure design and operating standards. However, the plant cryopreservation facility will require specific procedures for handling deposits of plant genetic resources. The FAO genebank standards and the operating principles used by CGIAR genebanks and other genebanks with cryopreserved collections, can inform the development of such procedures.

As mentioned in Section 4.3 above, it is proposed that there be a technical committee that oversees the technical standards of operation of the facility as well as technical guidelines for depositors.

4.4.1 Facility design, equipment and standards

The design and operation of a safety cryopreservation storage facility should adhere to established industry best practices and regulatory standards which define required facility design elements, staffing, quality systems, information technologies and operating procedures. ISBER 'Best Practices for Repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research (3rd Edition, 2011)' is considered an excellent reference document.

The facility should adhere to established physical standards for biorepositories, which would include uninterrupted power, security and access control systems, fire prevention and emergency preparedness. There must be guaranteed availability of the necessary specialized equipment, supplies and expertise to operate a low-temperature storage facility, including a reliable source of electricity and liquid nitrogen (e.g. capacity to generate liquid nitrogen on site), and appropriate liquid nitrogen storage systems for the collection. Back-up systems should include redundant capacity to replenish storage tanks with liquid nitrogen and adequate back-up storage capacity within the facility to manage any possible equipment failure with written procedures for transferring samples from a failed or malfunctioning

unit. The facility will need to have real-time environmental monitoring systems with continuous monitoring of all critical equipment. An alarm notification system will be in place with 24 hour / 7 day emergency response available. A programme for preventative maintenance and replacement of storage equipment essential to the functioning of the facility should be in place. Data and records management systems must provide for complete traceability of deposits and significant events such as receipt and return of deposits, sample transient warming, sample destruction and sample transfer within the facility. The electronic management system should have full audit capabilities and adhere to established data and access security standards.

Given the technical complexities of the facility and evolving nature of plant conservation, it is proposed that a stability programme be put in place for monitoring the performance of the cryopreservation protocols in maintaining viability and genetic integrity over time, as well the facility's performance in sustaining optimum storage conditions. It will be particularly important to track transient temperature fluctuations that could endanger the viability of the samples in transit and in storage.

The facility's technical committee, in association with its host and/or managing institution, would agree the industry standards to follow and keep them up to date in accordance with ISBER and other relevant standards. In addition, the technical committee would oversee the monitoring programme in association with the host/ managing institution and relevant depositors.

4.4.2 Technical guidelines for depositors

The samples that are deposited in the facility will need to be of the highest possible quality to ensure the successful regeneration – after what could be hundreds of years of storage – of healthy, vigorous plants that are 'true to type'. Providing depositors with guidelines on putting their material into cryopreservation and preparing and packaging samples for deposit will help ensure the quality of the conserved material. The facility's technical committee would agree on the guidelines for depositors and keep them up to date in accordance with FAO genebank standards, CGIAR standard operating procedures (SOPs) and other relevant standards. The committee will also need to keep abreast of improvements in the application of cryopreservation protocols to different crops.

To ensure that the deposits are received in the best possible condition, it is proposed that the facility provide the depositor with standardized labelling practices and specialized containers for packaging (a standard deposit box) and transporting (a dry shipper) the samples. The facility would also be responsible for organizing transport with the appropriate cargo airline to ensure the correct and efficient handling of the dry shipper.

Given the very lengthy storage timeframe provided by the facility, it will be desirable for documentation on the cryopreservation protocol and method for rewarming the samples and regenerating the plants to be stored at the facility. The documentation would need to be in paper as well as electronic form to ensure that it can be read in a hundred years or more. As with the samples, the documentation would be subject to the 'black box' agreement, with rights to the use of the documents remaining with the depositor.

4.5 Options for the location of a safety back-up cryopreservation facility

In contrast to the Svalbard Global Seed Vault, which benefits from the remoteness and natural climate of its location, a cryopreservation facility will require an institutional setting where the specialized equipment, supplies and expertise needed for its operation can be guaranteed. The Global Registry of Biodiversity Repositories (www.grbio.org) lists 7,449 institutions which hold biological collections and highlights the growing expertise and resources that exists to potentially support a plant genetic resources back-up cryopreservation facility.

In following the same principles that govern the Seed Vault, it can be foreseen that a government institution would be the facility's host from a legal and ownership perspective, and thus accountable for the obligations as set out in Section 4.3.1. However, a number of options could be considered by the host regarding the physical location of the safety back-up cryopreservation facility with, it is recommended, the management of the deposits vested with a plant genetic resources institute. The opportunities and challenges of each option are summarized in Appendix 8. Possible options include the following:

Option 1: A commercial biorepository with management provided by a plant genetic resources institute

> The enormous growth in commercial biorespository services creates the opportunity for a fee-for-service model to be used to support a back-up facility to preserve the biodiversity of vegetatively propagated plants and recalcitrant seeds. Commercial, for-profit organizations, such as BioReliance (a Sigma-Aldrich company), Fisher BioServices, BioStorage Technologies, Core CryoLab, Masy BioServices, BioKryo GmbH, and many others offer biostorage services to organizations. Oversight and coordination of deposits into a commercial biorepository would be best facilitated by a plant genetic resources institute that enters into a contractual relationship with a commercial biorepository service provider.

Option 2: A public biorepository with management provided by a plant genetic resources institute

> There are a large number of government funded / managed or public, not-for-profit biorepositories which could support the scale of storage services required of the plant genetic resources back-up cryofacility. Many regional hospitals, provincial/state health services, or national health services offer biobanks for the storage of human cell and tissue samples. Examples include the UK Biobank, the US National Cancer Institute's Biorepositories, the UK Stem Cell Bank or the Canadian Biosamples Repository. In addition, large, multi-cell / tissue, not-for-profit biobanks possess the capacity and expertise to support a plant back-up cryopreservation facility, including organizations such as the European Cell Culture Collection, or the Integrated Biobank of Luxembourg (IBBL). Oversight and coordination of deposits into a public biorepository would be best facilitated by a plant genetic resources institute, which would enter into a contractual relationship with the biorepository service provider.

Option 3: Co-locate the safety back-up cryopreservation facility with an "iconic" organization

The Svalbard Global Seed Vault has been important in enhancing global public awareness to the need for crop genetic resources conservation. The selection of a highly visible, publically recognizable, "iconic" location for the back-up cryofacility has the potential to bring further global awareness to the issues of food security and crop genetic resources conservation. Co-locating the crop cryofacility with an organization that has high public visibility, a global reputation in conservation biology and the necessary cryopreservation infrastructure could bring significant public awareness, fundraising, community education and advocacy opportunities for crop cryobanking. This could be a zoo or botanical gardens, such as The Smithsonian Conservation Biology Institute, the San Diego Zoo Institute for Conservation Research or the Royal Botanical Gardens at Sydney, Australia or Kew, UK.

Option 4: Utilize resources at an existing plant genetic resources cryopreservation facility

Several CGIAR Centers and a number of other crop genebanks around the world have the expertise and infrastructure required to perform long-term cryostorage of plant materials. Examples include Bioversity International (Belgium) and the International Potato Center, (CIP, Peru), Leibniz Institute of Plant Genetics and Crop Plant Research, (IPK,Germany), United States Department of Agriculture (USDA,USA), Rural Development Administration (RDA,Republic of Korea), National Agriculture and Food Resource Organization (NARO, Japan), among others.

Option 5: A new stand-alone cryopreservation facility

The new construction of a stand-alone cryopreservation facility for the safety back-up of vegetatively propagated and recalcitrant seed crops at an existing crop genebank is an option. However, the scale of the current demand for a safety back-up cryopreservation facility of 5,000 to 10,000 accessions does not warrant the construction of such a stand-alone cryostorage facility.

4.6 Costs of setting-up and running a safety back-up cryopreservation facility

Based on the responses from the survey of institutes with cryopreserved collections, it is expected that approximately 5,000 accessions could be initially deposited in the cryofacility, with a further 5,000 coming in over the following five years (Section 4.1). These numbers have been used to develop the cost estimations, assuming there are three replicate samples per accession. The samples will be sent to the cryofacility and stored in standard cryoboxes. A number of standard cryoboxes may be used in order to cater for the range of plant parts (tissue, budwood, embryos, etc.). For the purpose of these cost estimations, a standard cryobox that can hold 81 2ml vials (each vial equivalent to one replicate) has been used.

The cost of establishing a cryostorage facility is directly related to its location and administrative arrangement. If the facility is housed in an existing institute with a cryobank, (Options 2, 3 or 4, Section 4.5), it is estimated that an investment of US$430,000 will be needed to establish the facility and US$108,000 for its annual maintenance (See Tables 1 and 2). These estimates take into account equipment, labour, supplies and services costs, including overhead costs. Note that under any of the options to locate the facility in an existing institute, there may be the risk of having double administrative costs: one applied by the institute housing the physical facility, the other by the plant genetic resources institute managing the deposits. At the same time, it is possible that the institute housing the facility will have equipment available to rent, which will significantly reduce the costs of establishment (see Table 1). The cost estimations include a contingency for the potential increase in the capacity of the facility beyond the initial 10,000 accessions.

An endowment is a logical financing mechanism for a safety back-up cryofacility with a long-term perspective. Therefore, the in perpetuity costs have been estimated using a 4% interest rate (Appendix 7, Table 7-2). The rate of interest is used to estimate the present value of the future investments. Note that the interest rate of government institutions tends to be low, particularly in the Euro area (OECD data, 2017). A conservative range of values has been used to account for the potential variation (Appendix 7, Table 7-3). The investment return to the endowment would probably be higher than the interest rate for government institutions. Equipment replacement was accounted for based on the average life span of the equipment or the time interval for periodic expenses.

Table 1. Summary of costs estimations for establishing and maintaining a cryostorage facility in an existing institution (Options 2 to 4, Section 4.5)

Cost Components	Description	Set-up Costs (US$)	Annual Running Costs (US$)	In Perpetuity (US$, 4% Int. rate)
Equipment	Includes equipment needed to store about 10,000 accessions: cryotanks, LN Generator, pipe system, pressurized LN tank, office and monitoring equipment, cryocontainers	227,054.72	14,984.71	389,602.43
Supplies	Liquid Nitrogen, safety supplies and certification	9,562.03	5,562.03	148,612.79
Labor	Part time technician and part time supervisor	82,000.00	36,000.00	1,018,000.00
Services	Documentation system, data storing services, supervision and administration	111,501.59	45,741.09	1,268,370.75
SUBTOTAL		**430,118.34**	**102,287.83**	**2,824,585.97**
Contingency	To expand the collection (at a rate of 10,000 accessions every 10 years)	0.00	5,927.45	154,113.68
	TOTAL	**430,118.34**	**108,215.28**	**2,978,699.65**

To assess the potential costs of a fee-for-service model (Option 1), quotations were requested from commercial biorepositories for storing 10,000 accessions (Annex 4). The estimations recived, as shown in Table 2, only cover a basic storage fee, a removal fee and a contract fee. They do not cover the supervision and management costs of a plant genetic resources institute. Nor do the estimations include an increase in the deposits beyond the estimated 10,000 accessions expected by 2022.

Table 2. Cost comparison of renting storage space in a commercial cryobiorepository (Option 1, Section 4.5) and storing in a public institution managed by a plant genetic resources institute (Options 2 to 4)

Option/Biorepository Companies		Description	Set-Up Cost (US$)	Annual Running Costs (US$)
Option 1	Bio-Kryo, Germany	Covers only storage fee of the collections. 10-year contract. Accessions are managed within the company using an internal database system.		21,288.42
	Fisher Clinical Services, Switzerland	Covers only storage fee of the collections. 6-year contract. Accessions are managed within the company using an internal database system.	9,801.86	9,632.34
	Core Cryolab, Canada	Covers only storage fee of the collections. 5-year contract. Accessions are managed within the company using an internal database system.		19,013.62
Options 2, 3, 4		Assumes existent equipment in storing institution. Accounts for supplies (LN, safety equipment and part time labor of a technician, land rent and administration fees).	82,410.41	53,837.86

The estimated costs in Table 2 for Option 1, compared to Options 2 to 4, include only the costs associated with storage and documentation in the case of Options 2 to 4 in order to provide a more balanced comparison with the fee-for-service costs provided by the biorepository companies. The cost comparison in Table 2 indicates that housing the cryofacility in an existing institute is more expensive than contracting out to a commercial biorepository firm. The difference in costs is mainly due to the time allocated to technical staff in charge of managing the facility and to the administrative charges linked to this cost line. The higher costs of Options 2 to 4 should be considered as in some way accounting for a lower risk than using a commercial repository, given the uncertainty for a private biorepository to offer long-term (i.e. decades-long) service and possible concern of depositors in using a private, for-profit storage facilities.

To estimate the cost of a new stand-alone cryostorage facility (Option 5, Section 4.5), the costs of building a facility would have to be added to the previous estimation.

4.7 Recommendations

Based on the above, the Expert Group recommends that:

1. Action is taken to enhance the long-term conservation of crop collections of importance for food security that are vegetatively propagated or have recalcitrant seeds. Priority should be given to the safety back-up of existing cryopreserved collections and to furthering the cryopreservation of collections under Article 15 of the Plant Treaty and of crops on Annex 1. Given CGIAR Center obligations under Article 15 and on-going activities in crop cryopreservation, the Expert Group recommends that the CGIAR genebanks take the lead in following up on the recommendations of this feasibility study regarding a global initiative on crop cryopreservation and the setting up of a safety back-up cryopreservation facility.
2. A major global initiative is launched to accelerate the development and implementation of crop cryopreservation. This feasibility study has emphasized the advantages of cryopreservation for the long-term conservation of vegetatively propagated and recalcitrant seed crop collections. It has also highlighted the challenges faced in the timely and wide-scale implementation of cryopreservation to different crops and different collections with diverse genotypes. The Expert Group recommends a collaborative effort among researchers and genebanks that is focused on the specific technical and practical issues hampering the adaptation of cryopreservation protocols to different collections. The Expert Group believes that there is a compelling case for such an initiative to secure at-risk high priority collections. The study revealed that as many as 100,000 unique and distinct accessions of Annex 1 and Article 15 vegetatively propagated and recalcitrant seed crops are in high maintenance, costly and vulnerable field and *in vitro* genebanks. The Expert Group calls on the CGIAR to develop the project proposal in coordination with relevant partners and on donors to sponsor the initiative.
3. A cryopreservation facility is set up to accommodate the estimated 5,000 to 10,000 accessions arising from current, on-going cryopreservation activities at CGIAR and other genebanks identified in this study, that need safety backup over the coming five years. Although the physical capacity requirements are modest, the Expert Group emphasizes the importance of the facility being established and operated in accordance with the principles and best practice for a global cryofacility laid out in this report. Over time and if, as the Expert Group recommends, substantive effort and funding are applied to the cryopreservation of crop collections, the facility will need to grow in capacity and stature and take on a global role in providing safety back-up storage for the 100,000 plus accessions that could potentially be deposited by national and regional genebanks around the world.
4. The safety back-up cryofacility operates according to the same principles and policies that govern the Svalbard Global Seed Vault. Rights of ownership over deposits would remain with the depositing institute under a 'black-box' agreement. Furthermore, given the facility's 'in perpetuity' perspective, the Expert Group emphasizes the importance of its governance and funding being assured on a long-term basis. The Group concludes that as with the Seed Vault, this is best guaranteed through a host government commitment in the framework of the Plant Treaty and Crop Trust endowment mechanism with international oversight.

5. The design and operation of the cryofacility adhere to the best practices and regulatory standards in place for biorepositories. Although the facility's equipment requirements are modest (one storage container with a single back-up for the next five years, potentially increasing to 10 to 12 storage containers to hold 100,000 accessions), the Expert Group emphasizes the importance of ensuring that the infrastructure meets established industry standards and practices, given the high-tech specialised equipment of cryopreservation. It recommends that a technical committee be put in place that can oversee the standards of operation.
6. The management of deposits to the cryofacility be carried out by an institute active in plant genetic resources conservation. The physical infrastructure could benefit from location with an established biorepository. The study indicates that this could be the most cost-effective option.
7. The cryofacility act not only as an additional level of security for cryopreserved collections, but, if requested by a depositor, also as a direct back-up to field and *in vitro* collections. In the latter case, the collection would be entered into cryopreservation either by a third party or the depositing institute, and the facility would relieve the depositor of the burden of having to maintain a cryopreservation infrastructure for the long term. This service will be important in the absence of the proposed initiative to enhance the capacity for cryopreservation around the world.
8. The organisations commissioning this feasibility study agree a process to:
    a. Gather further inputs and reactions to this study as deemed necessary.
    b. Secure collections currently cryopreserved by CGIAR and other interested genebanks in a back-up facility with an initial capacity of about 10,000 accessions.
    c. Develop a proposal for a major global initiative on cryopreservation, and seek sponsorship for it.

5. STAKEHOLDER RESPONSE TO THE FEASIBILITY STUDY

This report has been shared with the 19 institutes that responded to the survey for feedback.

Ten institutes (CIAT, IITA, JKI Germany, EMBRAPA/CENARGEN Brazil, Luke Finland, CNR Italy, NARO Japan, CRI Czech Republic, InHort Poland, and INTA Argentina) provided feedback and the comments are compiled below. (Note, Bioversity and CIP are members of the Task Force and therefore, have provided inputs throughout the study.)

Overall, the feedback was very positive. Most of the responses mentioned that the report is clear, comprehensive and provides a very useful analysis on the state of cryopreserved collections worldwide, their potential future expansion and the need for a safety back-up cryopreservation facility.

The respondents found the recommendations clear and pertinent. The importance of having an international initiative to promote cryopreservation as a tool against the loss of valuable genetic resources and, in some cases, for virus elimination, was highlighted. Some respondents would like to share this report at a ministry level to promote the use of cryopreservation in their countries. There was a comment that the upfront investment required to get collections into cryopreservation seems to be an entry barrier for many institutes and hence should be the key intervention point targeted by for investment and by the plant cryopreservation community as a whole.

According to most of the respondents, the lack and insecurity of funds provided for cryopreservation activities as well as the difficulty of protocol transfer between laboratories and low plant regeneration after cryopreservation, are the major constraints that they are currently facing.

Some respondents agreed that existing cryopreserved collections need a secure back-up and that the recommendations provide a good coverage of the different aspects of setting-up a back-up safety storage facility, including the crucial aspects of host and depositor obligations.

APPENDICES

APPENDIX 1 - THE IMPORTANCE OF VEGETATIVELY PROPAGATED AND RECALCITRANT SEED CROPS

Many of the world's most important crops for food, nutrition and livelihoods are vegetatively propagated or crops with recalcitrant seeds. Below are some examples (in alphabetical order).

Apple (*Malus* spp.)

Is the most common and culturally important fruit crop of temperate areas. The genus *Malus* is characterized by large diversity, but thus far this has not been translated in the cultivated (*Malus sylvestris* var. *domestica*) varieties (Way et al. 1990). Modern commercial apple orchards are dominated by only a few cultivars and many breeding programs utilize only a few well known cultivars in crosses for commercial apple production (Noiton and Shelbourne, 1992; Way et al. 1990). The world annual production of apple constitutes 126 million tons (FAOSTAT) with major producers being Europe, USA and Canada.

Aroids

Include over 100 genera and 3750 species belonging to the Araceae family. Popular ornamentals, aroids are also one of the oldest cultivated crops in tropical Asia and America. It is the fourteenth most consumed vegetable worldwide. The most important cultivated aroid genus is Taro, *Colocasia esculenta*, which is used as food, but also for medicinal purposes and for animal fodder. Other important members of the edible aroids are giant taro (*Alocasia macrorrhizos*), swamp taro (*Cyrtosperma merkusii*), and arrowleaf elephant's ear (*Xanthosoma sagittifolium*). All aroid plant parts are edible, however these plants are mostly appreciated for their starchy roots whose carbohydrate content is similar to that of potatoes. Protein content in aroids is slightly higher than in potatoes, but more than double the amount contained in sweetpotatoes and cassava. Most of the global production 10 million tons per year (FOASTAT, 2014) comes from developing countries. Largest producers are Nigeria (3.5 million tonnes), China and Ghana (both 1.5 million tonnes), Cameroon and Papua New Guinea.

Banana and plantain (both *Musa* species)

Form the world's most important fruit crop, with an annual production of 144 million tonnes per year (FAOSTAT, 2014). Banana and plantain are cultivated in more than 100 countries spread over five continents in tropical and subtropical regions and provide a significant source of revenue for small-scale farmers all year round. Besides being a nutritious fruit containing large amounts of potassium and vitamins B6 and C, it is an important staple food crop for more than 400 million people living mainly in Africa (for example Uganda, Nigeria, Democratic Republic of Congo, Cameroon and Ghana) and Latin America (for example Colombia and Peru). Annual consumption of bananas in Uganda is the highest in the world, at 0.70 kg daily per person. In Uganda, East African Highland bananas belong to a specific genomic group within the genus *Musa*, and are consumed as a boiled puree of mashed bananas. Their importance is illustrated by the fact that these bananas are called 'matoke',

which is another word in the local language for food. The banana export trade in 2013 was estimated at 17 million tonnes, with Ecuador as the biggest exporter. This export trade represents only 15% of world banana production, meaning that 85% is produced for local consumption.

Cacao (*Theobroma cacao*)

Is grown in the tropics, with most of the production in a band within 8° from the equator, sometimes called the "Cocoa Belt". Cacao requires hot, moist conditions to grow and will not withstand prolonged drought conditions without seriously depressing the tree´s vegetative and reproductive functions. Some 95% of global cocoa production comes from several millions of small cacao growers who might have an average of some three hectares allocated to the crop with perhaps an annual yield of some 330 kg per hectare leading to their producing about one tonne of dried beans per annum (CacaoNet 2012). In total, more than 20 million people depend directly on cacao for their livelihood. In 2014, world production reached 4.5 million tonnes of cacao beans. Africa holds a dominant position with 1.3 million tonnes coming from the Côte d'Ivoire. Ghana and Indonesia (both 0.6 million tonnes) are two other important producers.

Cassava (*Manihot esculenta*)

Is the fourth most important supplier of food calories in the tropics. The principal economic product is starchy roots, which have a wide range of end uses, most notably including human food, animal feed, and industrial products. The main features of cassava that impact its form of utilization are its starch content, nutritional value, post-harvest storage characteristics, and toxicity. Cassava utilization typically performs five main roles: (1) famine reserve; (2) rural food staple; (3) urban food staple; (4) livestock feed and industrial raw material; and (5) earner of foreign exchange. World production in 2013 of about 276 million tonnes is the energy equivalent of 100 to 105 million tonnes of cereal grains (FAOSTAT). Cassava is an important component of traditional tropical cropping systems, particularly in subsistence and family farming, where intercropping with a variety of other staple or cash crops is the major production system. The rotation cultivation system with a fallow phase and intensive annual cultivation, are also used (Leihner 2002). Large plantations are becoming more common as the crop is industrialized, especially in Latin America (Brazil) and Asia (Thailand and Indonesia).

Citrus (*Citrus* spp.)

The most well-known examples of citrus fruits with commercial importance are oranges, lemons, limes, grapefruit, mandarins and tangerines. Although citrus fruits are grown all over the world in more than 140 countries, most of the crop grows on either side of a belt around the equator covering tropical and subtropical areas of the world. Yearly world production is 126 million tonnes (FAOSTAT, 2014) and world's most important producers are Brazil (20 million tonnes), China (15 million tonnes) and USA (12 million tonnes). With low protein and very little fat content, citrus fruits supply mainly carbohydrates, such as sucrose, glucose, and fructose. In addition to vitamin C, which is the most abundant nutrient, the fruits are a source of B vitamins (thiamin, pyridoxine, niacin, riboflavin, pantothenic acid, and folate), and contribute phytochemicals such as carotenoids, flavonoids, and limonoids.

Coconut (*Cocos nucifera*)

Is one of the most important palm crops in tropical and subtropical regions, cultivated on about 12 million hectares worldwide. Ten million farmers in developing countries are currently relying on the produce of this palm as an important source of nutrition and income. Widely recognized as the 'tree of life', coconut palm can be converted into a range of commercial and industrial products with nutritional and medicinal properties, as well as structural timber and resilient. Edible products include beverages, fresh kernel and are consumed locally, while refined products, including virgin oil, shell charcoal, husk fibre and cortex are exported. Additionally, coconut wood recovered from the older portion of the trunk provides timber components that are used in the production of furniture, handicrafts as well as building materials. Apart from the normal phenotype, unique coconut varieties possessing either a delicious jelly-like solid endosperm, or a flavoursome aromatic liquid endosperm, have been attracting great attention from customers and food producers on a global scale. In 2015 the world coconut production was 12.2 million metric tonness of copra equivalent, of which 10.5 million metric tonnes came from the Asia-Pacific region and representing 86% of the world production. Coconut oil is the major product traded at 2.2 million metric tonnes and mostly comes from the Philippines and Indonesia (75%). According to a recent report from Asia and Pacific Coconut Community, around 700 million palms need to be replanted each year in the next two decades.

Coffee (*Coffea* spp.)

Is produced in about 80 countries, mostly in Latin America, Africa and Asia, and provides a significant source of foreign earnings for nearly 125 million people (Osorio 2002; Musoli et al. 2009; Bramel et al. 2017). World coffee production is estimated at nine million tonnes annually (FAOSTAT) and has grown steadily over the past 50 years. However, it is now threatened by the continued rise of production costs, as well as problems related to negative impacts of climate change and higher incidence of pests and diseases (ICO 2014). Coffee genetic resources conserved *ex situ* in genebank collections are recognized as one of the major genepools that can be utilized to develop improved varieties with drought stress tolerances, pest and disease resistances, high cup quality, and increased production (Bramel et al. 2017).

Potato and sweetpotato (*Solanum tuberosum* and *Ipomea batatas*)

Are globally important tuber and root crops, respectively. Potato is the world's fourth most important crop and is grown in all global continents except Antarctica. More than a billion people worldwide eat potato and production exceeds 300 million tonnes annually. Sweetpotato is the seventh most important crop and is associated more with the tropical and subtropical zones of the world. Sweetpotato is important in providing nutrients to small holder farmers where it is grown and in 2016 four scientists (three from CIP) received the World Food Prize for their efforts in biofortified vitamin A sweetpotato in Africa. Both crops are seen as buffer crops in a climate change scenario as both will produce a crop in marginal soils with little inputs where other crops could not survive.

Yam (*Dioscorea* spp.)

Is a multi-species crop which constitutes a staple food for over 100 million people in the humid and subhumid tropics. Its world production accounts for 68 million

tonnes annually (FAOSTAT). *Dioscorea rotundata* and *D. cayenensis* (known as white and yellow Guinea yams, respectively) are the most important yams in West and Central Africa where they are indigenous, while *D. alata* (referred to as water yam) is the most widely distributed species globally. Several traits of *D. alata* make it particularly valuable for commercial cultivation. These include high yield potential, ease of propagation, early growth vigour for weed suppression, and long storability of tubers. Tubers possess a high nutritional content with an average crude protein content of 7.4%, starch content of 75–84%, and vitamin C content ranging from 13.0 to 24.7 mg/100g. Over 90% of world yam production occurs in the yam belt of West and Central Africa with Nigeria alone accounting for about 68 percent of the world's total (FAO, 2002).

APPENDIX 2 - CRYOPRESERVATION AS A LONG-TERM CONSERVATION METHOD

Critical aspects in the long-term conservation of plant genetic resources are the maintenance of the viability and the genetic integrity of the stored accessions. These aspects and the costs of cryopreservation versus field and *in vitro* conservation were investigated using both literature review and the experience of CGIAR genebanks.

1. Longevity and genetic stability of plant materials in cryopreservation

Preparation and storage of plant tissues at ultralow temperatures (cryopreservation) will expose such tissues to various chemical, physical and physiological stresses. Therefore, it has been argued that such treatments would cause tissue injury and possibly result in genetic change. To ensure that the genetic integrity of plants recovered from cryopreservation has not been compromised, studies have been undertaken to assess aspects of plant morphology, cell cytology and DNA constitution after cryopreservation. Some of the results of those studies are presented and discussed below.

An internet survey of scientific literature typing in the topics “genetic stability”, “cryopreservation” and “plant*” found 165 papers in peer reviewed journals combining these three topics. Many of them were reviewed by Harding (2004) and Volk (2010).

The results obtained from morphological assessments have been contradictory. For example, growth rate of rice (*Oryza sativa*) and potato (*Solanum tuberosum*) plants recovered from cryopreserved tissues was found to be lower than that observed in plants from non-cryopreserved tissues (Moukadiri et al. 1999; Harding and Staines 2001). However, in many other crops, no changes in growth rates were detected (Ryynanen and Aronen 2005; Sisunandar et al. 2010a). Karyotype studies showed that cryopreservation rarely inflicts gross changes in ploidy level (Helliot et al. 2002; Ryynanen and Aronen 2005; Urbanova et al. 2006; Sisunandar et al. 2010a). At the genomic DNA level, microsatellite markers showed that plants produced from cryopreserved tissues showed patterns similar to their non-cryopreserved counterparts (Harding and Benson 2001; Richards et al. 2004). Meanwhile, cryopreservation has been shown to cause significant changes in global DNA methylation patterns in several species (Harding et al. 2000; Johnston et al. 2009).

Of the 28 papers published since the beginning of 2015, 18 dealt with the application of genetic markers to detect whether differences between cryopreserved and non-cryopreserved plant material. These 18 papers cover a variety of plant species (including potato, tomato, chrysanthemum, grape) and tissues (meristems and cell suspensions), and use different techniques (three use AFLPs, five use ISSR, nine use RAPDs, one uses SCoT and three use biochemical markers, one DNA methylation and two ploidy measurements). Only two of these papers (both published in journals not rated by The Thomson Reuters Impact Factor) observed some differences but they could not link them to phenotypic differences. All others did not report any difference between cryopreserved and non-cryopreserved material using either genetic markers or phenotypic observations.

Since cryopreservation aims at long-term storage, the studies on plant tissues retrieved from cryobanks after over ten years of conservation are of particular interest. Among papers reviewed, five papers had assessed the effects of >10 years storage of plant tissue in liquid nitrogen, and all of them confirmed the retention of morphological, biochemical and genetic traits in cryopreserved materials. For example:

- Transformed hairy roots of *Hyoscyamus muticus* were successfully cryopreserved for 16 years without loss of transgene expression (Hakkinen et al. 2016).
- Alfalfa cell cultures showed physiological, biochemical and ploidy stability after 27 years of storage (Volkova et al. 2015).
- Shoot tip tissue cultures of wasabi stored in liquid nitrogen for 10 years were compared to cultures stored *in vitro* for 10 years. The paper concludes that cryopreservation is a superior conservation method compared to *in vitro* in maintaining genetic stability for the long-term storage of wasabi germplasm (Maki et al. 2015).
- Strawberry and pea explants were able to regenerate into normal plants after 28 years of storage in liquid nitrogen (Caswell and Kartha 2009).
- Dormant buds of apple stored in liquid nitrogen showed no changes in the percentage viability after 10 years. Interestingly, some samples showed an increased viability (Volk et al. 2008).

Another putative concern could be the mutations caused by background radiation during the very long (over 100 years) storage. To our best knowledge, there were no studies in plants on this topic. However, studies on mouse embryos, exposed to the equivalent of 2000 years background radiation, did not show any genetic variation (Glenister and Lyon 1986).

The overwhelming outcome from the reviewed studies is that the more recently developed methods of cryopreservation do not cause genetic or epigenetic changes, thus suggesting that the plant tissues passed into and through cryopreservation would remain genetically stable over time.

This is in contrast with data on the genetic stability of plant materials maintained for the long-term in *in vitro* collections, where somaclonal variation (spontaneous mutations) have been often reported and tend to increase over time (see e.g. Bairu et al. 2011).

2. Costs

The scientific literature on the costs of plant genetic resources conservation is relatively recent and limited. Table 2-1 below presents a summary of studies documenting the costs of the three main conservation methods used for vegetatively propagated and recalcitrant seed crops.

Cost comparison is not easy for several reasons. First, the cost data corresponds to different years of evaluation with the oldest reference referring to 1990 values and the most recent to current 2017 values. Second, the estimations do not necessarily include the same cost components, with many of them failing to include administration costs that could be more significant than expected. Third, the values for cryopreservation are often based on research situations where a small number of

accessions are manipulated rather than routine operational use for large genebank collections. This is important because the cost calculations are on a per accession basis. Fourth, in some cases the values are just projections based on a hypothetical number of accessions and not on the real number of accessions stored, specifically in the case of cryopreservation costs. Lastly, the costs refer to different crops that have different physiologies. It is not possible to compare annual with perennial crops, monocots with dicots, or cultivated with wild materials.

Taking the points above into consideration, the studies summarized in the table below, indicate that introducing an accession into cryopreservation is expensive and more expensive than introducing an accession into in vitro conservation or maintaining it in the field. Garming et al. (2010) for instance reported that putting a banana accession into cryopreservation costed annually $1,833.98 per accession while putting it into *in vitro* was about $1,217.59 per accession. For elm (Harvengt et al. 2004), the costs of establishing a clone in cryopreservation ($37.6) was five times more expensive than putting it in the field ($7.6). However, maintaining an accession in cryopreservation tends to be considerably lower than conserving it using any of the other methods, particularly when a larger number of accessions are manipulated (Keller et al. 2013). A striking example is the case of coffee. Dulloo et al. (2009) reported that the annual cost of maintaining an accession in the CATIE field genebank in Costa Rica was $1,523, but maintaining the accession cryopreservation was only $3. IPK in Germany also reports cheaper maintenance costs for cryopreservation ($6.4 – 12) than field conservation ($49 - 59) in the case of potato collections (Schäfer-Menuhr et al. 1996; Keller 2006; Keller et al. 2008).

In cases such as wild apple, storing budwood tends to be more cost effective than storing shoot tips. Volk et al. (2010) found that using dormant budwood as cryopreserved materials of two apple species, *M. sieversii* and *M. orientalis*, was significantly cheaper than using shoot tips. The establishment costs to put budwood into cryopreservation totaled $42 with an annual maintenance cost of $1.63, while costs of using shoot tips was about $1,500 for the establishment and $0.27 for maintenance.

Table 2-1. Comparative cost of establishing and maintaining an accession in cryopreservation compared to in the field or in *in vitro*

Institution	Crop	Year of reference	Cost per accession (E: Establishment, M: Maintenance)			Source
			Cryopreservation	Field	In vitro	
CATIE	Coffee	2009	E: $55 M: $3 (Assumed 2000 accessions)	E: $69.62 M: $1,523 (Based on 1992 accessions)		Dulloo et al. (2009)
CacaoNet	Cacao	2016	E: $500 M: $4 (Based on estimations from experience UK Reading)	M: $126 (Assumed 2,500 accessions in a global collection)		CacaoNet (2017) (personal communication) CacaoNet (2012)
IPK	Potato and related species	2002	M: $6.4 – 12 (Shoot tip is conserved)	M: $49 – 59		Schäfer-Menuhr et al. (1996) Keller (2006) Keller et al. (2008)
	Garlic	2010	E: Starting from – Bulbil/inflorescences: $418 – *In vitro* plants: $561 M: No. Accessions – 100 acc.: $27.24 – 500 acc.: $5.45 (all costs accounted)	E/M: $61.8 (Annually planted, core collection 54 accessions)		Keller et al. (2013)
CIAT	Cassava	2004		M: $5 (Based on 6000 accessions)	M: $4.20 (Based on 6000 accessions)	Reed et al. (2004) Based on Roca (personal communication)
	Cassava	2009	E: $61.92 (Based on 640 accessions, research level)		M: $ 24.34 (Based on 6592 accessions)	Global Public Good project (2010)
USA (no detail on the institute)	Sweetpotato	1990		$28 (Based on 1000 accessions)	$22 (Based on 1,000 accessions)	Reed et al. (2004) Based on Jarret and Florkowski (1990)

Institution	Crop	Year of reference	Cost per accession (E: Establishment, M: Maintenance)			Source
			Cryopreservation	Field	In vitro	
USA (no detail on the institute)	Temperate Fruit tree germplasm	1999	E: $50-75 M; $1 (M costs includes only the costs of LN)			Reed et al. (2004) Based on Hummer and Reed (1996)
ITC -Leuven/ Bioversity	Banana	2009	E: $1,833.98 M: $7.37 (Energy and LN, comprehensive estimation)		E: $1,217.59 M: $192.43	Garming et al. (2010)
AFOCEL (Association Forêt Cellulose)	Elm	2004	E: $ 37.6 M: $ 0.14 /tube (Cost include labor and consumables, conserve 200 buds per clon)	E: $7.6/clon producing plant, €9.5 / clon for establishing the clonal archive M: $11.4 / clon/ year Replace the materials every 15 years		Harvengt et al. (2004)
USDA-National Plant Germplasm	*M. sieversii* and *M. orientalis*	2010	Dormant budwood, E: $42, M: $1.63 Shoot tips, E: $1,500, M: $0.27 Seeds, M: $0.22 Pollen, M: $0.27			Volk et al. (2010)
CIP	Potato	2009		M: $57.61 (Includes facility and overhead charges)	M: $12 (Includes facility and overhead charges)	Global Public Goods project (2010)
	Potato and Sweetpotato	2017	Potato E: $280/accession (based on 450 accessions) M: $7.1 (based on 2,500 accessions) Sweetpotato E: $1,680, M: $7.1 (Based on 2,500 accessions)			D. Ellis (personal communication)

APPENDIX 3 - STATE OF CRYOPRESERVATION OF VEGETATIVELY PROPAGATED AND RECALCITRANT SEED CROPS*

** Appendix 3 is not included in the public version of the report based on the request of some data providers*

APPENDIX 4 - ASSESSMENT OF FIELD AND *IN VITRO* COLLECTIONS

National and regional genebanks around the world have important collections of vegetatively propagated and recalcitrant seed crops that are not the target for cryopreservation at this time. These collections are held in the field or *in vitro* and not safe from natural or human hazards. They can be expected to become the subject of cryopreservation in the future, in particular should there be a global initiative of advancing the development and implementation of cryopreservation, as recommended by this feasibility study.

In order to assess the potential future requirement for the safety back-up cryopreservation facility, data were gathered on these collections. The data gathering focused on crops important for food and agriculture in Annex 1 and under Article 15 of the Plant Treaty. The sources of information included the FAO State of the World Report on Plant Genetic Resources for Food and Agriculture, FAO World Information and Early Warning System (WIEWS), the genebank information system Genesys, Crop Trust project documents and crop strategy reports and the websites of individual plant genetic resources institutes.

The data are compiled in Table 4-1, below. The data do not include the field and *in vitro* collections held by the 15 institutes that provided information on cryopreserved collections in the survey (Appendix 3).

Table 4-1. Collections of vegetatively-propagated and recalcitrant seed crops held only in field and in vitro genebanks. The crops are those featuring in Annex 1 and Article 15 of the Plant Treaty.

Crop	Crop type	No. accessions in *ex situ* collections	Some major holding institutes
Apple	Clonal	59,922	GEN, USA VIR, Russia NIAS, Japan
Banana and plantain	Clonal	11,606	CIRAD, France CARBAP, Cameroon BPI, Philippines
Breadfruit and relatives	Clonal	3,158	SPC, Fiji USDA, USA NTBG, USA
Cassava	Clonal	36,529	ICAR, India NRCRI, Nigeria SAARI, Uganda
Citrus	Clonal	36,410	CCSM-IASP, Brazil NIAS, Japan CRI, China
Coconut	Recalcitrant seeds	1,680	CPCRI, India PCA, Philippines IPRI, Indonesia

Crop	Crop type	No. accessions in ex situ collections	Some major holding institutes
Jerusalem artichoke	Clonal	544	IFVC Novi Sad, Serbia USDA, USA NGRC, Sweden
Major aroids	Clonal	14,696	WLMP, Papua New Guinea MARDI, Malaysia NBPGR, India
Potato	Clonal	98,285	INRA, France VIR, Russia NR6, USA
Strawberry	Clonal	12,027	USDA, USA PGRC, Canada VIR, Russia
Sweetpotato	Clonal	35,478	NIAS, Japan S9, USA MHRP, Papua New Guinea
Yam	Clonal	15,903	UAC, Benin PGRRI, Ghana UNCI, Côte d'Ivoire
Cacao	Recalcitrant/ Intermediate seeds	23,107	INIAP, Ecuador MCB, Malaysia CRU/UW, Trinidad and Tobago
Coffee	Recalcitrant seeds	30,483	IAC, Brazil ECICC, Cuba JARC, Ethiopia
Total number of accessions		**379,828**	

APPENDIX 5 - CHALLENGES IN PLANT CRYOPRESERVATION

1) Challenges in genebank capacity/operations

The survey to assess the state of cryopreservation asked the institutes about the major issues that limited their abilities to cryopreserve accessions. They were asked to select among a set of pre-determined options: insufficient budget, lack of skilled personnel, lack of equipment, protocol issues, and to specify other issues, if any. The responses were summarized in the Section 3.4.

Twelve out of 20 institutes listed insufficient funding as the major constraint to implementing cryopreservation. Protocol issues and lack of skilled personnel were mentioned by five and ten institutes, respectively. Only three institutes considered lack of equipment as the major issue. Some mentioned specific issues, for example, insufficient plant number in the collection to perform cryopreservation (IPK, Germany), lack of space in the old building (CIAT) and variability among accessions in response to cryopreservation (IITA).

Further analysis of the responses demonstrated that the majority of surveyed cryobank facilities suffered from only one or two major constraints. The CGIAR Centers actively engaged in cryobanking (Bioversity and CIP) apparently have sufficient funds to maintain their cryogenic facilities and their annual rate of cryopreservation at its current level, but they are in need of skilled personnel and additional support to further develop cryopreservation protocols for poorly responding crops. CATIE Costa Rica, University of Oulu Finland and CRI Czech Republic stated that they possess both expertise and equipment, as well as working cryopreservation protocols, and require only funds to enhance cryopreservation. IRD France and CNR Italy indicated that they lack both funds and personnel.

These results suggest that the expertise and operational protocols for some crops already exist among the group of institutes that are already engaged with plant cryopreservation, in particular at CGIAR and European genebanks. However, they have a great need for funds to support the further implementation of cryopreservation and to enhance the skills of staff to be able to perform cryopreservation at high efficiency and with uniformity of the results.

The constraints in budget, infrastructure and personnel are especially acute for NARIs in developing countries and limit even their ability to conserve collections even by traditional methods. This is particularly critical for collections of vegetatively propagated and recalcitrant seed crops since they are often kept solely in the field where the risk of loss of diversity can be great. Only a substantive initiative on capacity-building and support for cryopreservation would enable many NARIs to be depositors of collections to the back-up cryopreservation facility.

2) Challenges in protocol development and implementation

Based on the abundance of literature on plant cryopreservation, particularly for the plants that are the most important for food and agriculture, one may assume that cryopreservation methods for the majority of staple crops are well-developed. However, this assumption is not always correct. Before discussing this

point further, it may be useful to draw the line between the terms used in this report: the “method” and the “protocol”. The method is usually more general as it represents the basic principles of material treatment to make it tolerant to extreme low temperature. The name of the method often indicates how plant material is treated during its preparation for cryopreservation. This may involve desiccation by air flow (“desiccation method”), exposure to series of cryoprotective solutions (“vitrification method”), using the foil strips to immerse material in liquid nitrogen (droplet-vitrification methods), etc. On the other hand, the protocol sets precisely the parameters of various treatments, i.e. in vitrification method. The protocol will describe the composition of cryoprotectant solutions, duration and temperature at which they should be used, etc. It is not uncommon that the same *method* is applied to various crops, but the *protocol*, i.e. the precise combination of treatments and their parameters, may vary greatly between different crops and even between accessions.

One of the profound challenges that cryobanks face is that cryopreservation protocols are species - and even genotype - specific. In practice, this means that a cryopreservation protocol developed and well-adapted to one crop often cannot be used for cryopreserving another crop without preliminary optimization. For example, the protocol used for potato cannot be directly applied for sweetpotato or banana, although the same method, “droplet-vitrification”, is followed in all cases. The three crops taken as an example – potato, sweetpotato and banana – require different compositions of culture media before and after cryopreservation, different combinations of cryoprotective treatments and different handling. Thus, in order to adapt the same method to various crops, the protocols often need to be optimized or adjusted to a certain level. The same problem exists when one protocol is applied to multiple accessions. For example, some potato accessions (less than 10% of the screened collection) do not respond well to the routine protocol applied at the CIP cryobank. Optimization and validation of the existing protocol for those accessions will require additional labor and funds and is currently left for the future.

A very similar challenge may be faced when attempting to adapt the protocol that is efficiently used in another cryobank for the same crop. For example, the protocol for cryopreserving the entire national garlic collection developed at RDA, Republic of Korea could not be adapted at IPK, Germany due to the issues with material contamination and poor response of the accessions specific to this geographic area.

For some crops, the cryopreservation protocols are not developed at all or result in very poor plant regrowth (<20%). Examples of such crops are breadfruit (all cryopreservation attempts have been unsuccessful), sweetpotato (plant regrowth after cryopreservation is often below 30%), coconut (difficulty in getting uniform starting materials, slow regrowth), some Andean crops (*in vitro* culture before cryopreservation is not optimized), and some others.

The reasons for such difficulties often lie in little understanding of stress physiology and stress tolerance for such plants that often originate from tropical regions. More efforts are needed to develop reliable and effective cryopreservation protocols for such crops.

3) Time, skill and cost requirements to put materials into cryopreservation

Cryopreservation entails a major use of resources, in terms of time, skills and costs. The period of time needed to put one accession into cryopreservation varies across crops and genotypes (see Tables 5-1 to 5-4). To cryopreserve a potato meristem, from a first multiplication cycle (four in total) until the accession is finally transferred to a cryotank and to safety back up tank takes about 18-19 weeks. A similar procedure with only three multiplication cycles takes 24-32 weeks in the case of sweetpotato.

The time period needed to put an accession into cryopreservation depends greatly on the standard operating procedures (SOPs) as well on the health and safety standards in place in the cryofacility. In the case of banana, Bioversity, for instance, conducts three independent successful repetitions with a 95 % probability that at least one plant can be regenerated from the stored material (Panis, personal communication). This includes multiplication of plant material, culture media preparation and cryopreservation. From receipt of two *in vitro* plants from the *in vitro* collection until three successful experiments are executed it takes on average 13 months, assuming that the process is performed by skilled, trained staff. A technician needs to be able to excise 15 small apical meristems from banana per hour without damaging them. A technician requires time to develop this *in vitro* practice skill. Longer period of times and the requirement of qualified labor translate into higher cryopreservation costs. This is the main reason why per accession costs of introducing one accession into cryopreservation are often high - as opposed to maintenance costs - and are not meaningfully affected by the increase in the number of accessions manipulated (see Table of comparative costs). Using the same example of banana cryoconservation, the establishing cost reported by Garming et al. (2010) was US$1,833.98 per accession, while the maintenance cost was only US$7.37.

IPK in Germany follows Dussert et al. (2003) for the three main crops cryopreserved in this facility: potato, garlic and mint. In order to work with well-growing, as well as on the poorly growing materials, IPK staff use two times 100 explants of the storage samples with additional control samples of two times 50 explants to record the regeneration rates. One of these two storage samples goes to a safety storage place in another town. In garlic the preparation is more complicated and requires a longer time span. Therefore, only 2 x 50 explants with 2 x 25 explants as controls are used. Only in cases when the regeneration is lower than 30% the staff takes other 2 x 50 explants to reach the same figure as it is for potato and mint (Keller 2017, personal communication). The time needed to cryopreserve potato in IPK is shown in Table 5-4.

Table 5-1. Cryopreservation steps and time period for potato, CIP

Description of protocol (step by step)	Current duration of each step (Dec. 2016)
First multiplication cycle (test tubes; stem segments with two axillary buds)	2-3 weeks
Second multiplication cycle (test tubes; stem segments with two axillary buds)	3 weeks
Third multiplication cycle (magenta vessels; stem segments with two axillary buds)	2 weeks
Final multiplication cycle (deep petri dishes; only apical buds)	3 weeks
Shoot tip excision, cryoprotection, freezing in LN2, thawing for viability assessment	1 day
Recovery cycle, intermittent and final evaluation	8 weeks
Transfer of accessions to cryobank and safety back-up tank	1 day
TOTAL	**18-19 weeks**

Source: CIP 2017

Table 5-2. Cryopreservation steps and time period for sweetpotato, CIP

Description of protocol (step by step)	Current duration of each step (Year 2016)
1st multiplication cycle (test tubes; explant stem segments with 2–3 axillary buds)	7–9 weeks
2nd multiplication cycle (test tubes; stem segments with 2–3 axillary buds)	7–9 weeks
Final multiplication cycle (test tubes; stem segments with 2–3 axillary buds)	1.5–2 weeks
Shoot tip excision and pre-culture on sucrose rich medium (24 hr)	1 day
Cryoprotection, freezing in LN, thawing for viability assessment	1 day
Recovery cycle, intermittent and final evaluation	8–12 weeks
Transfer of accessions to cryobank and transitory safety back-up	1 day
TOTAL	**24–32 weeks**

Source: CIP 2017

Table 5-3. Cryopreservation steps and time period for banana, Bioversity

Description of protocol (step by step)	Initial duration of each step (Year 2013)
Multiplication cycle until first material can be transferred onto regeneration medium	5-7 months
Regeneration of 54 plants + 40 multiplying cultures	1-2 months
Cryo 1	1 day
Regeneration of 54 plants + 40 multiplying cultures	1-2 months
Cryo 2	1 day
Regeneration of 54 plants + 40 multiplying cultures	1-2 months
Cryo 3	1 day
Regeneration of 54 plants + 40 multiplying cultures	1-2 months
Cryo 4	1 day
Evaluation of last experiment	2 months
TOTAL	**11–17 months**

Source: Panis, B. (personal communication)

Table 5-4. Cryopreservation steps and time period for potato in IPK, Germany

Description of protocol (step by step)	Initial duration of each step (Year 2009, DMSO droplet method)
Media preparation	1 h
Correctness and quality check, when the material (1 tube with *in vitro* microtubers) comes in from the *in vitro* facility (it is in the North of Germany in Groß Lüsewitz and the material is sent either by post of by visiting colleagues)	15 min
First multiplication step	30 min
Material grows then in an incubator	7-14 days
Second multiplication step	40 min
Material grows again in an incubator	7-14 days
Third multiplication step	25 min
Forth multiplication step and preculture start	1.5 h
Explant dissection	3 h
Overnight storage in Petri dish	
Preparation of the conservation step (documentation etc.)	30 min
Introduction into tubes with liquid nitrogen	2 h
Placement of the tubes into the cryotank	30 min
Preparation of the regeneration control sample	30 min
Cultivation of the regeneration control samples	8 – 12 weeks
Counting the survival and regeneration results (two times: survival after 3-4 weeks; regeneration after 8-12 weeks)	40 min
TOTAL	**700 minutes staff time needed to work on the accessions plus 14-15 weeks for the material to grow and develop.**

Source: Keller, personal communication

APPENDIX 6 - ADVANCING THE CRYOPRESERVATION OF CROP COLLECTIONS

This feasibility study has highlighted the potential of cryopreservation for the conservation of plant genetic resources but also the challenges in its application to crops that are vegetatively propagated or have recalcitrant seeds.

To take advantage of the effects of low temperature and to successfully store plant germplasm for extended periods using cryopreservation techniques, damage to the cells during freezing and thawing must be minimized. Over the last century, enormous progress has been made in understanding the basic elements responsible for low-temperature injury in cells and in the development of effective techniques to protect plant germplasm from cryoinjury. The challenges in implementing cryopreservation are discussed in Appendix 5. In summary, the reasons why large scale applications of cryopreservation to vegetatively propagated crops and crops with recalcitrant seeds are limited, are manifold. There are the financial aspects; not only an investment is needed to buy equipment (cryotanks, liquid nitrogen generators, laminar flow benches,...), but especially the transfer of plant tissues into cryopreservation is very labour intensive (and thus very costly). Last but not least there are the "technical aspects" that interfere with large scale applications. Here we can distinguish different cases; (i) crops for which response to different cryopreservation techniques are hardly investigated, (ii) crops for which successful cryopreservation was reported but only on a few accessions, (iii) crops for which current existing cryopreservation protocols did not result in acceptable post-thaw plant regeneration frequencies, irrespective of the accession.

There are a number of "next steps" that are required to advance cryopreservation for the safe conservation of the genetic diversity of major crops covered under the Plant Treaty's Annex 1 and Article 15 that have to be vegetatively propagated or have recalcitrant seeds. To help achieve this goal, a number of efforts can be undertaken including the following:

i. new, more advanced cryopreservation methods (such as using the "cryoplates", Yamamoto et al. 2012; 2015) allowing for better recovery and easier manipulation of the material;

ii. improved techniques for cryopreserving somatic embryogenic cell and callus cultures;

iii. cryopreservation methods that can be easily adapted to a wider range of genotypes, including new genotypes (such as the systematic approach using alternative cryoprotectant solutions (Kim et al. 2009; Popova et al. 2015);

iv. new technique to test for genetic fidelity in the recovered plants;

v. improved techniques to transfer plants recovered from cryopreserved materials into soil;

vi. improved techniques to detect and eradicate endogenous bacterial contamination. Such contamination is often invisible (but present) during regular tissue culture practices but often becomes problematic after cryopreservation.

There are many field collections that are coming under threat from a number of sources and that need to be put into cryopreservation as a matter of some urgency. For example, the present day coconut field conservation collections around the globe face many challenges, including those caused by the spread of major pests and incurable diseases, such as the phytoplasma lethal yellowing and viroid-caused cadang-cadang diseases. In addition, many field collections are under threat from cyclones, storms and tsunamis, and also by age, with productivity for breeding purposes declining steadily after 35 years. At this point in time, the Pacific Regional Coconut gene bank at Madang in PNG (the guardian and principal source of germplasm for that region) is under urgent imminent threat from an insect-borne phytoplasma, the Bogia Coconut Disease (http://www.cogentnetwork.org/bogia-syndrome-disease). So, this important collection must be moved immediately, and cryopreserved to protect it into the longer term.

Going forward with an initiative to cryopreserve the major livelihood and food security crops (Article 15 plus Annex 1 crops), will need collaboration among institutes working on crops in common, infrastructure development and capacity building and significant investment into the wide scale implementation of cryopreservation to priority crop collections. Collections at most risk from pest and diseases, natural hazards and budget constraints, should be given priority.

The following are the examples of major crops whose collections are conserved in *in vitro* conditions and the field, or just in the field, and that would greatly benefit from an initiative to develop and implement cryopreservation.

Cacao

Techniques used to introduce cacao accessions into *in vitro* culture as somatic embryos for the purposes of multiplication, distribution and cryopreservation are rather different to those already widely used for other vegetatively propagated crops (CacaoNet 2012). Somatic embryos currently represent the most appropriate target propagules for preservation of cacao germplasm (Li et al. 1998, Maximova et al. 2002, Fang et al. 2004). The approach can limit the transmission of Cocoa Swollen Shoot Virus (CSSV). Research is ongoing into improving the applicability of somatic embryogenesis techniques for a wider range of cacao genotypes. To our knowledge, Nestle (R&D in Tours, France) and UK University of Reading's International Cocoa Quarantine Centre (ICQC-R) are the only institutions that have cryopreserved some cacao genotypes. The use of secondary somatic embryos in a high concentration sucrose pre- culture and Plant Vitrification Solution 2 (PVS2) is currently used to cryopreserve frequently requested clones at ICQC-R. While by 2010 only 12 such accessions were cryopreserved by this institution, the aim was to back-up 10% of the ICQC-R collection within three years (Adu- Gyamfi 2011, CacaoNet 2012). We do not have current references on the number of accessions cryopreserved after that. Assuming that the method is successful, it will still need to be tested for larger number of genotypes. Advancing cryopreservation of cacao will require re-establishing the contacts with ICQC-R and developing a solid plan on implementation of their method, including testing it for multiple accessions available in the collection.

Cassava

Since the 1990's several techniques have been developed for cassava cryopreservation which resulted in various plant recovery percentage due to

genotypes. Cryopreservation of shoot tips of cassava using controlled and rapid freeze were developed at CIAT (Escobar et al. 1997; Escobar and Roca, 1997). However, this procedure is complicated and time consuming and generally gives low rates of recovery growth. From 2000, new cryogenic techniques, vitrification and encapsulation-dehydration (ED) were presented. The ED was adjusted for cassava (Manrique, 2000; Charoensub, 2004) and tested in an exploratory way in CIAT's core collection (10% of the global collection, consisting of 640 clones) (Escobar 2005). This study allowed the obtaining of a broader picture of clonal behaviour, by forming groups according to the performance in the freezing phase (Escobar, 2005). A minimum response of 30%, measured as full plant recovery, is considered to maintain a clone in a cryobank. Twenty-five percent of the core collection showed recalcitrance to these conditions. To overcome the cassava recalcitrance, the droplet-vitrification (DV) method has been recently tested, allowing a recovery of around 78% of the clones considered recalcitrant by the DE method (Escobar et al. 2014). Thus, to date, the most successful method for cryopreservation is DV, which is being adopted by CIAT as a routine system to establish a cassava cryobank with most of the clones from the *in vitro* collection.

Coconut

In the 1980s, the first attempt to cryopreserve coconut tissues was undertaken with immature zygotic embryos and using a chemical dehydration followed by a slow freezing technique (Bajaj 1984). More recently attention has shifted towards using mature zygotic embryos and using a physical dehydration method (Sisunandar et al. 2014); or using plumular tissues excised from mature zygotic embryos and using a chemical dehydration method (Sajini et al. 2011). The outcomes of the early work were relatively high in terms of recovery of viable tissues but very few plantlets were ever produced. However, by using a physical flash dehydration of embryos approach (Sisunandar et al. 2010b) up to 40% of plants in soil have now been achieved for 10 accessions. It was also shown that this cryopreservation method did not induce any measurable genetic change in the recovered plants (Sisunandar et al. 2010a). Establishment of plants in soil following cryopreservation of coconut embryos has only been reported using the physical dehydration approach of Sisunandar et al. (2010b) and the chemical dehydration approach of Sajini et al. (2011). To date these protocols have not been applied to any *ex situ* collections held in any genebank. In conclusion the 1,680 coconut cultivars held at the five international and numerous regional germplasm banks, and that are currently stored in the field, could be cryopreserved provided the techniques available are optimized on further cultivars and that necessary funding, training, equipment is also available

Coffee

Coffee conservation is mainly done in field genebanks, with a limited use of complementary approaches such as the cryopreservation of seeds, embryos or other tissue. The most successful cryopreservation method for coffee was developed by Dussert and Engelmann (2006) and included desiccation of seeds in 81%RH followed by slow precooling of seeds before immersion into liquid nitrogen. This method was applied at CATIE to 63 genotypes currently held in the USDA cryobank with average germination rate above 85% (William Solano, personal communication). CATIE stated that they have sufficient expertise and are willing to continue cryopreserving their core coffee collection core collection of about 100 accessions and 30 accessions of rare genotypes if the funds and equipment are been provided.

Potato

If one bases the results from the survey as representative of the state of the art of cryopreservation in crop plants, potato with 42% of the total crop accessions cryopreserved, appears to have had the most success in cryopreservation. At CIP, the production is likely the highest scale of cryopreserved plant collections with ca. 450 accessions cryopreserved in each of the last two years with increases in average recovery rates and decreases in contamination. The method used is a modification of the droplet vitrification method with success reported only as regeneration of whole, morphologically normal looking plants with roots (Vollmer at al. 2016). A cold acclimation step prior the shoot tip excision has been shown to be an important component of the CIP protocol. CIP has also initiated a strict quality control system where viability of cryopreserved accessions is assessed one or more years after cryopreservation which in a sample of 849 accessions, 99% still met standards after one of more years. Currently the CIP protocol is successful for 90% or more of the accessions attempted. The approach of large-scale potato cryopreservation developed and implemented at CIP may be adapted in other insittutions that are planning to cryopreserve their potato collections in the nearest future. Training of cryopreservation personnel from such institutions in protocol implementation, workflows and quality management systems would be the logical next steps.

Sweetpotato

While cryopreservation in potato is very routine, cryopreservation of sweetpotato lags far behind. Much research has gone into sweetpotato and there are numerous reports of great success with cryopreservation, yet none of these methodologies or successes can be repeated to data at CIP. It is feasible that methods were developed for breeding lines or selected genotypes that, when applied to a larger collection, do not work. It is also possible that the previous reports looked only at survival and not regeneration into a fully functional plantlet that could be moved into a greenhouse and further grown. The current challenge in sweetpotato is that cryopreservation works and can yield very suitable short-term survival in the formation of an apparent bud subtended by a rosette of leaves. Unfortunately, recovery of plantlets from this rosette of leaves is poor. The focus at CIP over the past year is to define parameters pre - and post - cryopreservation that contribute to enhanced shoot growth after cryopreservation. As there is a strong genotype influence over the ability to form elnongated plantlets post-cryopreservation, CIP is currently utilizing a labor intensive method of prescreening a small number of shoot tips from each accession and then doing a full cryopreservation run with those that do form elongated plants. In this way, a sweetpotato cryobank is being built with minimum quality standards while experimentation to develop a routine cryopreservation method for larger numbers of accessions is underway.

Taro

The cryopreservation of taro was initiated in 1996 (Takagi et al. 1997). The method was further developed and optimized at KU Leuven (Belgium) and successfully applied to 18 diverse genotypes from the *in vitro* collection of the Secretariat for the Pacific Community (SPC, Fiji) with high rates of plant recovery ranging from 73-100% (Sant et al. 2006; 2008). In 2008-2011, the KU Leuven/Bioversity International

with the financial support of the Crop Trust* worked on optimizing/developing protocols for all edible aroids, including taro (*Colocasia esculenta*), giant taro (*Alocasia macrorrhizos*), swamp taro (*Cyrtosperma merkusii*), and arrowleaf elephant's ear (*Xanthosoma sagittifolium*) using 13 accessions from SPC collection. Optimization of cryopreservation protocols and plant regeneration conditions resulted in recovery of 50-100 % for *Alocasia*, 50-70% for *Xanthosoma* and 20-40% for *Cyrtosperma*. Therefore, it can be concluded that the cryopreservation protocol for taro is developed and is ready to be tested for large-scale using multiple accessions. It is recommended that efforts are made to apply the developed protocols to cryopreserve 758 taro cultivars from Asia-Pacific countries at the Regional Germplasm Centre (RGC) of SPC in Suva, Fiji that are currently stored under *in vitro* conditions and in the field, providing required funding, training, and equipment.

* Crop Trust project "Development and refinement of cryopreservation protocols for the long-term conservation of vegetatively propagated crops".

APPENDIX 7 - COSTING OF A SAFETY BACK-UP CRYOPRESERVATION FACILITY

An estimation of the potential cost of the cryostorage facility is presented in Table 7-1. The cost estimation of the cryostorage facility covers establishment and running costs. Starting the cryostorage facility involves investment in equipment (capital goods or fixed costs) as well as investment in supplies (laboratory, office), infrastructure (database, capability to handle shipments), labor and services (administration fees, land rent). Once the facility is in operation, the annual expenses would be mainly recurrent investment in variable goods (supplies, labor services). One-time costs like the development of the management and inventory software or the certification of the facility account for only a part of the establishing cost.

The monetary values given to each item of the different cost components (equipment, supplies, labor, services) are based on information requested from providers, or assumptions based on best available information and correspond to current values. The equipment considered for this cost information is based on the operation of well-equipped, high-tech cryostorage facilities. We could not account for the effect of future technological development on the total maintenance costs. Liquid nitrogen (LN) is a critical supply. Access to a guaranteed and continuous source of LN is key to maintain the stability of maintenance costs . In terms of labor, at set-up, the facility would employ a full-time technician and partial time of a senior scientist. This time commitment would reduce significantly after the facility has been established.

The main cost items envisioned as part of services are expenses related to the development of the documentation software, land rent and administration costs, including overheads. This last item varies significantly across locations and institutions and could have a significant impact on the final budget. We assumed a mid-range overhead value of 15%. The budget includes also costs for potential travel expenses for the cryostorage facility staff to monitor/supervise shipments. Finally, the use of the facility could potentially expand to include a broader type of crops and type of materials conserved. Therefore, we assigned a budget line to account for this development. Based on these assumptions the cryofacility requires an investment of about US$430,118.34 for its establishment and US$108,215.28 for its annual running costs if it in housed in a existing facility.

Since a suggested financing mechanism for the cryofacility is the development of an endowment fund, the estimation of the in-perpetuity cost is relevant. We accounted for equipment replacement taking into account the average life spam of the equipment or the time interval for periodic expenses. Table 7-2, presents the in-perpetuity costs estimating using a 4% interest rate. The rate of interest used to estimate the present value of the future investments. Note that the interest rate of federal institutions tends to be low particularly in the Euro area (OECD data, 2017). We use a conservative range of values to account for the potential variation (See Table 7-3). While the investment return to this fund would probably be higher than the interest rate of the federal institutions, the use of a lower interest rate is a practical way to account for the inflation effect.

Table 7-1. Cryofacility Estimated Costs

Item		Description	Unit Cost in US$	Set Up Costs + 1 Year of Operation		Annual Running Cost (aprox 10,000 accessions)		In Perpetuity (using interest rate)
				Units	US$	Units	US$	4%
EQUIPMENT	Cryo tank	Capacity 30000 cryo-vials (2-ml)	33,540.00	1	33,540.00	1	1,865.02	48,490.54
	Back up tank	Capacity 30000 cryo-vials (2-ml)	33,540.00	1	33,540.00	1	1,865.02	48,490.54
	Racks for Cryotank		1,487.00	2	2,974.00	2	165.37	4,299.67
	Pipe system to supply nitrogen	Depends on the scale of the cryobank	25,000.72	1	25,000.72	1	1,390.19	36,144.85
	Generator	LN generator	100,000.00	1	100,000.00	1	5,560.59	144,575.25
	Office equipment	Computers, printers, desk	10,000.00	1	10,000.00	1	2,159.88	56,156.78
	Monitoring systems	Combined and monitoring system for 2 cryotanks	5,500.00	1	5,500.00	1	305.83	7,951.64
	Dry shippers	2 for boxes, 1 for bags	4,000.00	3	12,000.00	3	1,422.59	36,987.28
	Pressurized LN tank	230L tank	4,500.00	1	4,500.00	1	250.23	6,505.89
SUPPLIES	Liquid nitrogen supply	Expense/tank per year	2,527.90	2	5,055.80	2	5,055.80	131,450.80
	Safety supplies	Globes 1 pair	174.41	2	348.82	2	348.82	9,069.22
		Mask 1 package of 5	78.71	2	157.41	2	157.41	4,092.77
	Certification for the facility	One time cost	4,000.00	1	4,000.00			4,000.00
LABOR (LOCATION SPECIFIC)	Technician time, Set up	Reception, storage, monitoring, documentation, shipping, maintenance	50,000.00	1	50,000.00			50,000.00
	Technician time, recurrent		50,000.00			0.4	20,000.00	520,000.00
	Senior scientist, Set up	Overlook of operations	80,000.00	0.4	32,000.00			32,000.00
	Senior scientist, recurrent		80,000.00			0.2	16,000.00	416,000.00

	Item	Description	Unit Cost in US$	Set Up Costs + 1 Year of Operation		Annual Running Cost (aprox 10,000 accessions)		In Perpetuity (using interest rate)
				Units	US$	Units	US$	4%
SERVICES (LOCATION SPECIFIC)	Land rent	Includes services like internet access and electricity	1,341.60	12	16,099.20	12	16,099.20	418,579.20
	Documentation	One time cost. Software development	17,000.00	1	17,000.00			17,000.00
	Database license	One time cost. For storing information	6,000.00	1	6,000.00			6,000.00
	Support annual	plus back up support	2,700.00	1	2,700.00	1	2,700.00	70,200.00
	Data storing + servers	Monthly access 24/7	300.00	12	3,600.00	12	3,600.00	93,600.00
	Supervision	Travel cost	10,000.00	1	10,000.00	1	10,000.00	260,000.00
	Administration	Overhead costs-establishment	374,015.95	0.15	56,102.39			56,102.39
		Overhead costs-annual running	88,945.94			0.15	13,341.89	346,889.16
Sub-TOTAL					**430,118.34**		**102,287.83**	**2,824,585.97**
Contingency (growing number of accessions)		Assuming a growth of 10,000 accessions every 10 years	50,000.00				5,927.45	154,113.68
TOTAL					**430,118.34**		**108,215.28**	**2,978,699.65**

Table 7-2. In Perpetuity costs of establishing and maintaining the Global Cryofacility using a 4% interest rate

Item		In Perpetuity (using interest rate) 4%
EQUIPMENT	Cryo tank	48,490.54
	Back up tank	48,490.54
	Racks for Cryotank	4,299.67
	Pipe system to supply nitrogen	36,144.85
	Generator	144,575.25
	Office equipment	56,156.78
	Monitoring systems	7,951.64
	Dry shippers	36,987.28
	Pressurized LN tank	6,505.89
SUPPLIES	Liquid nitrogen supply	131,450.80
	Safety supplies	13,161.99
	Certification for the facility	4,000.00
LABOR (LOCATION SPECIFIC)	Technician time, Set up	50,000.00
	Technician time, recurrent	520,000.00
	Senior scientist, Set up	32,000.00
	Senior scientist, recurrent	416,000.00
SERVICES (LOCATION SPECIFIC)	Land rent	418,579.20
	Documentation	17,000.00
	Database license	6,000.00
	Support annual	70,200.00
	Data storing + servers	93,600.00
	Supervision	260,000.00
	Administration	402,992.55
Sub-TOTAL		**2,824,585.97**
Contingency (growing number of accessions)		154,113.68
TOTAL		**2,978,699.65**

Table 7-3. Effect of Interest Rate on the In Perpetuity costs

Rate of interest (%)	Total In-Perpetuity (US$)
1	10,579,989.31
2	5,506,502.69
4	2,978,699.65
6	2,143,247.83

APPENDIX 8 – OPTIONS ANALYSIS FOR THE LOCATION OF A SAFETY BACK-UP CRYOPRESERVATION FACILITY

Option 1: A commercial biorepository with management from a plant genetic resources institute

Opportunities: The advantage of using a commercial biorepository service is that they adhere to international best practices and utilize state-of-the-art storage and informational management systems. Successful commercial biorepositories have sustainable cost models which ensure their long-term viability. Due to the size of many of the large commercial biorepositories, there is an economy of scale which allows these organizations to be cost competitive compared to a small stand-alone operation. Large international commercial biorepository companies can offer multiple locations and management of virtual inventories which could reduce shipping costs.

Challenge: The financial stability of commercial biorepository services could affect the ability for a company to be able to offer a long-term (ie decades) commitment which could entail costly transfer of the deposits to another facility if the commercial entity shuts down operations. National institutes may have issue with depositing their collections into private, for-profit storage facilities due to concerns over access, changes in corporate ownership, or long term costs. The commercial biorepository may or may not be located in close proximity to the managing institute which may create some communication / coordination issues.

Option 2: A public biorepository with management from a plant genetic resources institute

Opportunities: Contracting with an existing facility would significantly reduce the infrastructure and equipment requirements required to set-up the crop back-up facility. Government support of an established biorepository could provide the needed political and financial stability required to establish a safety back-up cryopreservation facility for vegetatively-propagated and recalcitrant seed crops.

Challenge: Compliance with biorepository best practices may be an issue if sufficient funding and resources are not provided by the government or not-for-profit agency. The commercial biorepository may or may not be located in close proximity to the managing institute which may create some communication / coordination issues, in particular with regard to transportation. Double costing of administration costs is also a potential constraint.

Option 3: Co-locate the safety back-up cryopreservation facility with an "iconic" organization

Opportunities: Leveraging the co-location with an existing high-profile conservation organization could be used to increase public awareness of the issues facing plant genetic resources management. A crop cryo-facility can help enhance the well-established public awareness, community education and advocacy initiatives of the conservation organization, for the benefit of both initiatives. Opportunities for local economic development through eco-tourism and education can be used to offset costs.

Challenges: Compliance with biorepository best practices may be an issue if insufficient funding and resources are not provided through public / private sources. "Consumer fatigue" may result in the need for renewal of public exhibits and education programs to continue to attract resources to the program. Priorities for funding campaigns may not favor investment into a long-term "invisible" cryopreservation safety back-up facility after the novelty of the project wears off. The potential for increased overhead and / or administration costs is also a constraint.

Option 4: Utilize resources at an existing plant genetic resources cryopreservation facility

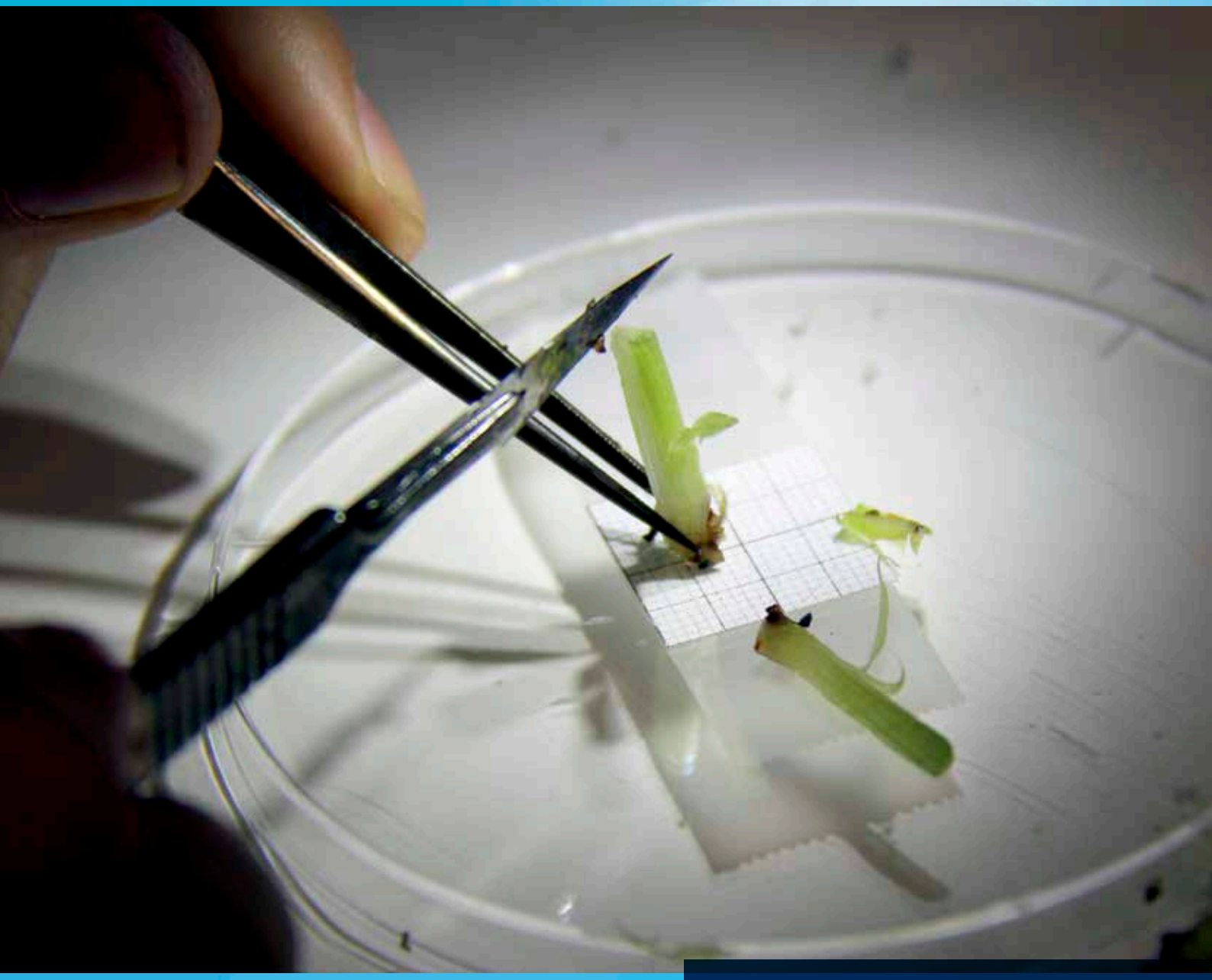
Opportunities: Investment in enhancing the cryopreservation infrastructure and capabilities of one or more of the existing CGIAR genebanks or other crop genebanks would be seen as a positive contribution to existing efforts. Commitments to the Plant Treaty are already in place for CGIAR Centers and the host governments of other crop genebanks which would expedite setting up the facility. CGIAR or other crop genebanks already have a long-term commitment to plant genetic resources conservation and hence are well situated to "understand" a long-term commitment.

Challenges: There may be costs associated with expanding the capacity and ensuring the existing infrastructure has the redundant equipment, monitoring systems and data management tools required to operate the back-up facility. Compliance with biorepository best practices may be an issue if sufficient funding and resources are not provided to the initiative.

Option 5: A new stand-alone cryopreservation facility

Opportunities: Identifying a location for the facility would not be limited to existing countries / locations where crop genebanks exist. The construction of a new facility could coincide with the establishment of a national / international genebank that currently does not exist.

Challenges: There would be a need for significant financial resources to establish and operate a stand-along plant genetic resources cryopreservation facility. Compliance with establishing and maintaining biorepository best practices may be an issue if local expertise cannot be established or if insufficient long-term funding and resources are not provided.



ANNEXES

ANNEX 1: TERMS OF REFERENCE FOR THE FEASIBILITY STUDY AND MEMBERS OF THE EXPERT GROUP AND TASK FORCE

1. Feasibility Study for a Global CryoVault: Terms of Reference for External Experts

Summary:

Crop genetic diversity is vital to ensure our current and future food security. Without it, farmers cannot adapt to climatic changes and make agriculture more productive, resilient and sustainable, and breeders cannot develop new and improved varieties. A key element of plant conservation good practice is to hold materials in genebanks or seed banks (conserved as seeds, vegetative shoots *in vitro* or cryopreserved materials) that are also backed-up in another location. The global crop conservation community is part of the way there: the Global Seed Vault, in Svalbard, Norway, has the largest back-up collection of seeds originating from the majority of countries around the world.

But what about crops that are not conserved through seeds, like bananas, potatoes and cassava? They are currently mainly conserved as collections of field plants or plantlets in test tubes – a relatively expensive, time-intensive conservation method in the long term that also can lead to mutations in the materials being conserved. Therefore cryopreservation (or long-term storage at ultra-low temperature) programs are in the process of being established. However, for these vegetatively propagated crops, whose annual global production is estimated to be more than one billion tonnes and worth at least US$ 100 billion annually (estimate based on FAOSTAT), there is no global back-up collection. These crops need a global back-up system – a Global CryoVault.

As a vital complementary follow-up step to the Global Seed Vault, Bioversity International is leading an effort in partnership with the other CGIAR Centers, KU Leuven and the Crop Trust to identify a location or locations to host the Global CryoVault. The Global CryoVault will store a back-up collection of vegetatively propagated crops held in collections managed by CGIAR centers and other international, regional and national genebanks that request such a service. The materials in the vault are meant to replace catastrophic losses of genetic diversity of crops that cannot be secured in conventional seed genebanks (see “Needs assessment’ below).

Before embarking on this ambitious plan, Bioversity/KU Leuven with International Potato Center (CIP) and the CropTrust plan to work together to commission a study to assess the technical and political feasibility of establishing a Global CryoVault. A cross-institutional Task Force is established to support external experts implementation of the study’s and closely liaise with the experts.

Scope:

Key issues to be addressed by external experts through this feasibility study include the political context, the location, the governance and the financing model for a

Global CryoVault. The study will also cover any auxiliary services that the sites might provide, for instance training or conferencing.

Timeframe and Level of Effort:

There is available a six-month period from 1 December 2016 to 31 May 2017 for the Task Force to arrange this study by a group of up to five globally-recognized independent experts who will implement the study. The envisaged level of effort is 20 days per external expert member, approximately four to five working days per month over four month period.

Profile of experts:

A multidisciplinary team of External Experts is envisaged, of up to five individuals, representing key expertise relevant to technical, economic and policy of global crop conservation practices. It is foreseen that the team will select its Chair and comprise independent consultants. While the engagement over time will be with up to five External Experts consultants, the study will conclude with a virtual broader consultation, comprising 10 to 12 further independent experts to vet the report's findings.

The role of the CGIAR / CropTrust task force will be to establish with donors these Terms of Reference, identify and engage the External Experts (under individual consultancy contracts administered by Bioversity International) and serve as resource to the External Experts. Depending on the topic and need, representatives of the Task Force may be asked to make themselves available to attend Expert meetings. In the workplan for the overall deliverable, there will be a series of interactions between the Expert team and the Task Force.

It should be stressed that the confirmation of the External Experts nominated by the Task Force is subject to donor review.

Independence and impartiality measures:

The world of plant cryopreservation is relatively small. Consequently, the Task Force recognizes that some of the External Expert Group may have current or past professional affiliations with one or more of the potential host sites. To maintain impartiality, numerous mitigating measures will be effected:

1. None of the External Expert Group will be current employees of CGIAR or CropTrust.
2. Global diversity in External Expert Group members will serve as a check against any one member promoting a specific site without due evidence.
3. The Task Force will support, guide and oversee work of the External Expert Group, flagging any potential conflicts of interest for deliberation, initially to the Chair or, if necessary, to all of the External Expert Group.
4. The External Expert Group will select its Chair.
5. Given the Task Force comprises CGIAR and CropTrust staff, the External Expert Group Chair must have a well-established and long-term independent affiliation (i.e., outside of CGIAR and CropTrust).
6. The structuring of this study furthermore mitigates against conflict of interest through the establishment of the triple-pronged mechanism comprising –

a. Task Force of CGIAR and CropTrust
b. External Expert Group of three to four independent experts
c. Expert consultative process to vet the draft recommendations comprising another 10 or so experts

7. Donor community review of the full list of external experts

Areas of inquiry:

1. Needs assessment

a) A literature review and expert consultation will provide the documented business case and technical specifications for the safety back up in cryopreservation. The requirements of international agricultural research centers of the CGIAR (and the plausible development of future requirements) should be given priority with respect to the total volume of accessions of vegetatively propagated crops requiring backup (and specification of crops). The potential of a CryoVault to serve as a safety back-up for sexually propagating crops that produce recalcitrant (desiccation intolerant) seeds, seeds with limited longevity at -20°C or even orthodox seed will be also considered. The review will assess the needs for quality management and standards of practice necessary for acceptance of materials for long-term safety duplication and how they might be attained.

b) An exploration of the relative costs and benefits of safety back-up in one location (one CryoVault, similar to one SeedVault) versus safety back up in multiple locations (scattered safety back up of vegetatively propagated crops) or at a commercial/ industrial cryostorage facility.

2. Comparison of locations

a) A relative comparison of individual locations for safety back up (if in a single location).
While Bioversity International has proposed, together with KU Leuven, locating the Global CryoVault in Leuven, Belgium, there are other potential sites. For instance, the USDA Fort Collins facility holds cryopreserved safety duplicates, as do RDA in South Korea and IRD in France. CIP, IITA and CIAT manage cryostorage facilities in Peru, Nigeria and Colombia respectively. China, India, Japan, Mexico and Brazil might host potential locations to manage cryostorage. Shortlisting four to five viable sites will be part of the task of the external experts.

Such a comparative desk study should examine:

1. Willingness of the host country/location to have and commit to hosting a long-term Cryovault
2. Cost effectiveness for set up and maintenance per site of the cryopreserved safety back-up
3. Capacity to manage total number of accessions
4. Phyto-sanitary issues and controls in the country for the list of crops
5. Ability to handle Annex 1 and non-Annex 1 crops
6. Local / regional security considerations

7. Risks and costs of ensuring building safety from natural or other disasters (bunker, etc.)
8. Quality assurance capacities in the site
9. Whether policies in the country (including access and benefit sharing policies) could affect the Cryovault's ability to receive, use and distribute materials in the execution of its responsibility
10. Logistical aspects to access and secure the proposed site
11. Established legal status of hosting facility (status as international or regional or national, and relative merits)
12. Experience of host institute with backing up cryopreserved plant material
13. Political, legal and potentially financial support of the host country
14. Ability to export (ship back to origin genebank) cryopreserved materials when requested.

Note: The feasibility of the potential site for organizing complementary services such as international training courses and workshops may be also taken into account with the remark that those services are not compulsory and may be hosted by a different location / institution.

3. Financial sustainability considerations

A financing model for the establishment and operation of the CryoVault will include identifying potential investors, embracing also the private sector, and potentially considering more than one financial scenario, or a phased approach. The study will address three basic costing scenarios, low-mid-high cost, with limited cost checks. Low is safety back-up only with basic technical assistance. Mid is safety back-up with medium level technical assistance limited to routine cryopreservation protocols. High is safety back-up, robust technical assistance (protocol tailoring and optimization activities), and training/capacity building services.

4) Institutional and political setting

a) Assessment of the relevance and influence of international agreements (Plant Treaty, Nagoya Protocol, Cartagena Protocol, etc).

b) Potential governance and management structure of the facility. Assessment of the roles and interest of the Crop Trust, the CGIAR, advanced research institutions, national governments who are not party to the ITPFRFA, or Nagoya Protocol or Cartagena Protocol, etc in being involved.

c) Assessment of the overall commitment and interest of institutes to prepare a costed proposal to host the facility among the locations identified in Part 2(a) above.

Indicative workplan with milestones: Exact timing to be agreed between Task Force and expert panel taken into account that (i) the Draft report is prepared by 15 April and (ii) the final feasibility study is presented by 31 May.

Weeks*	1	2	3	4	5	6	7	8	9	10	11	12
First interaction with Task Force (brief)	x											
Identification of chair and work process	x											
Outreach and document collection		x										
Establishing the scope of study (i.e. countries to include in comparative analysis)			x									
Second interaction with Task Force				x								
Write initial findings				x								
Virtual consultation with broader group of experts (meeting, through asynchronous email exchange, through individual discussion)					x	x						
Consultation with Task Force							x					
Share draft report									x			
Task Force review										x	x	
Final report delivered to Task Force												x

* Tasks do not need to be delivered in consecutive weeks; i.e., the weeks may be distributed over more than a three-month period

Deliverables:

- Draft report prepared by 15 April with 15 day period for remarks from Task Force members prior to broader consultation.
- Consultation of a range of 10 to 15 key stakeholders (through individual or group consultation process, as determined by the Experts) with associated documentation of such.
- Final feasibility study report with recommendations produced for review among the interested partners and donor community by 31 May.
- NB: It is foreseen that the study will identify multiple potential locations and that the top candidate locations will then be asked to develop costed proposals for the development, hosting and management of the Cryovault. The development of the detailed costed proposals is outside of the scope of this consultancy. Nevertheless, to deliver this consultancy basic cost data for potential sites will be needed.

2. Members of the Expert Group

Dr Jason P. Acker
Professor
Laboratory Medicine and Pathology, University of Alberta
President of the International Society for Cryobiology
Senior Research Scientist, Canadian Blood Services
Canada

Dr Stephen Adkins
Professor
School of Agriculture and Food Sciences,
Centre for Plant Science, QAAFI
The University of Queensland
Australia

Dr Alfredo Alves
Plant Physiologist
Senior Research Scientist
International Affairs Team Leader
EMBRAPA Cassava & Fruits
Brazil

Dr Daniela Horna
Agricultural Economist
Independent Consultant
Germany

Ms Jane Toll
Retired
Former Senior Project Manager at the Global Crop Diversity Trust
UK

3. Members of the Task Force

Dr David Ellis
Genebank manager
International Potato Center
Peru

Dr Michael Halewood
Senior Scientist, Policies, Institutions and Monitoring Team Leader
Bioversity International
Italy

Mrs Charlotte Lusty
Head of Programs
Global Crop Diversity Trust
Germany

Dr Bart Panis
Senior Scientist, Cryopreservation
Bioversity International
Belgium

Task Force Chair
Dr Elena Popova
Genebank Program Scientist
Global Crop Diversity Trust
Germany

Dr Nicolas Roux
Senior Scientist, *Musa* Genetic Resources Team Leader
Bioversity International
France

ANNEX 2: SURVEY QUESTIONNAIRE AND LIST OF RECIPIENTS

1. Introductory letter to the Survey

Subject: Feasibility study for a Back-up Cryopreservation Facility

Dear ...

The Svalbard Global Seed Vault (SGSV) serves as the ultimate safety back-up storage facility for seed crop collections. For crops that cannot be stored as seed, cryopreservation offers a long-term conservation option, which your institute, the CGIAR Centers and other genebanks around the world are increasingly opting to use. However, there is no back-up storage facility akin to SGSV for cryopreserved collections. Therefore, Bioversity International, the International Potato Center (CIP) and the Global Crop Diversity Trust have initiated a study on the feasibility of establishing an ultimate safety back-up cryopreservation facility for crops that cannot be stored in SGSV.

The feasibility study is being undertaken by an Expert Group supported by Bioversity, CIP and the Crop Trust, and funded by the Australian Centre for International Agricultural Research, the Swiss Agency for Development and Cooperation and the German Federal Ministry for Economic Cooperation and Development. The study focuses on species that are important for food and agriculture and that are vegetatively-propagated or have recalcitrant seed. As is the case for SGSV, rights of control over materials deposited in the cryopreservation facility would remain with the depositing institutes, governed by a 'black box' agreement between the depositors and the cryopreservation facility and subject to international oversight. In determining the feasibility of such a venture, the opinions, advice and assistance of genebanks with cryopreserved collections are paramount. The Expert Group, therefore, would be very grateful for your contributions to the study.

Our first task is to assess the need for a safety back-up cryopreservation storage facility. We are seeking information on the scale of cryobanking, currently and planned over the next five years, for vegetatively-propagated and recalcitrant seed crops. We would greatly appreciate your help in assembling this information by completing the survey attached below. Also attached is the list of institutes to which we are sending this survey. Should you know of others we should contact, please let us know.

As we move forward with the study, we hope to seek your inputs on other questions as well. We will endeavour to keep such requests limited. However, as mentioned earlier, your views and help are critical to this feasibility study and we believe you will agree with us that securing the diversity of vegetatively-propagated and recalcitrant seed crops warrants due attention and care.

With kind regards,

The Expert Group

2. Survey questionnaire

1. Contact information
Institute:
Contact: Name & Position
Email & Tel:

2. Information on collections of vegetatively-propagated and recalcitrant seed crops that are already cryopreserved and are undergoing routine cryopreservation over the next five years (the cryo-protocols are fully operational for most accessions/ genotypes).

Please list crops by genus and species

Genus and species	Number of accessions currently in the collection and how they are stored				Projected total number of accessions in cryopreservation by 2022 (assume continuing adequate budget and any expected expansion of the collection)	Material cryopreserved
	Total	Field	*In vitro*	In cryo		
Example: Allium sativum	2,456	2,456	2,120	1,200	1,800	Shoot tips

3. Other collections of vegetatively-propagated and recalcitrant seed crops, and the state of development of their cryopreservation.

Genus and species	Number of accessions currently in the collection			Is a cryopreservation protocol under development or have you plans to source/develop a protocol over the next 5 years?	Where applicable, indicate how many accessions you may be able to put into cryopreservation by 2022
	Total	Field	*In vitro*		
Example: Ipomoea batatas	1,500	1,500	1,000	Plan to have a working protocol by 2019	Approx..80
Example: Manihot esculenta	1,270	1,270	560	No protocol available	Not applicable

4. Duplication of cryopreserved collections
For the crops that are cryopreserved routinely at your genebank (listed under 2 above), please indicate if any part of the cryopreserved collection has been duplicated (backed-up) off-site at another cryobank.

Genus & species	Number of cryopreserved accessions safety duplicated off site in cryopreservation	Host of the cryopreserved duplicate (Organization and country)	Type duplication agreement
Example: Allium sativum	230	USDA, USA	Black box

5. Potential use of a safety back-up cryopreservation facility
If an international cryostorage facility became available in 2018 and your institute agreed to use it, would you have accessions with sufficient sample replicates ready to make a deposit?

Genus & species	No. of accessions with sufficient replicates available to make a deposit in 2018	No. of accessions that could have sufficient sample replicates to make a deposit by 2022

6. For the crops listed in 2 and 3 above, what constraints are you currently facing and expect to face over the coming five years, that limit the rate at which you can cryopreserve accessions and produce sufficient sample replicates? Please select relevant issues from the list below and indicate where they are crop specific.

□ Insufficient budget

□ Lack of skilled personnel

□ Lack of equipment

□ Protocol issues

□ Other technical issues *specify* _____

□ Other issues *specify* _____

3. Survey recipients

#	Institute	Country	Response
1	Instituto Nacional de Tecnología Agropecuaria (INTA)	Argentina	Completed the survey
2	Bioversity International *	Belgium	Completed the survey
3	Embrapa Genetic Resources and Biotechnology (CENARGEN)	Brazil	Completed the survey
4	Chinese Academy of Agricultural Sciences, Institute of Crop Sciences (CAAS/ICS)	China	Completed the survey
5	College of Horticulture, Northwest A&F University	China	Completed the survey
6	International Center for Tropical Agriculture (CIAT)*	Colombia	Completed the survey
7	Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)	Costa Rica	Completed the survey
8	Crop Research Institute (CRI)	Czech Republic	Completed the survey
9	Centre for Pacific Crops and Trees, South Pacific Community(CePact/SPC)	Fiji	No response
10	Department of Biology, University of Oulu	Finland	Completed the survey
11	Natural Resources Institute (Luke)	Finland	Completed the survey
12	Institute of Research for Development (IRD)	France	Completed the survey
13	Julius Kühn-Institut (JKI), Institut für Züchtungsforschung an Obst	Germany	Completed the survey
14	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Genebank Department	Germany	Completed the survey
15	Tissue Culture and Cryopreservation Unit, National Bureau for Plant Genetic Resources (NBPGR)	India	No response
16	Trees and Timber Institute (IVALSA), National Research Council (CNR)	Italy	Completed the survey
17	Genebank Project, Genetic Resources Center, National Agriculture and Food Research Organization (NARO)	Japan	Completed the survey
18	World Agroforestry Centre (ICRAF)*	Kenya	Completed the survey
19	Centro Nacional de Recursos Genéticos, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (CNRG/INIFAP)	Mexico	No response
20	International Institute for Tropical Agriculture (IITA)*	Nigeria	Completed the survey
21	International Potato Centre (CIP) *	Peru	Completed the survey
22	Department of Plant Genetic Resources of Horticultural Crops, Research Institute of Horticulture (InHort)	Poland	Completed the survey
23	Rural Development Administration (RDA)	Republic of Korea	No response
24	N. I. Vavilov Institute of Plant Genetic Resources (VIR)	Russia	No response
25	Millennium Seed Bank, Royal Botantic Gardens, Kew	UK	No response
26	National Laboratory for Genetic Resources Preservation (NLGRP), USDA	USA	Responded with the decision not to participate. Will not use the facility.

* CGIAR genebanks

ANNEX 3: AGENDA AND PARTICIPANTS OF THE BONN MEETING

Feasibility Study for a Safety Back-up Cryopreservation Facility
Joint meeting of the Expert Group and the Task Force
AGENDA

List of participants

EXPERT GROUP

1. Jason Acker (University of Alberta, Canadian Blood Services, Canada)
2. Stephen Adkins (University of Queensland, Australia)
3. Alfredo Alves (EMBRAPA, Brazil)
4. Daniela Horna (Independent Consultant, Germany)
5. Jane Toll (Independent Consultant, UK)

TASK FORCE

6. Bart Panis (Bioversity International, Belgium)
7. David Ellis (CIP, Peru)
8. Michael Halewood (Bioversity International, Italy) – remotely
9. Nicolas Roux (Bioversity International, France)
10. Elena Popova (Global Crop Diversity Trust, Germany)

OBSERVERS FROM THE CROP TRUST

11. Charlotte Lusty
12. Luigi Guarino

Dates: 15-16 May, 2017

Location: Crop Trust Headquarters in Bonn, Germany

Background

The Svalbard Global Seed Vault (SGSV) serves as the ultimate safety back-up storage facility for seed crop collections. There is no back-up facility like SGSV for the crops that are vegetatively propagated or have recalcitrant seeds, even though hundreds of millions of people depend on them for food and livelihoods. These crops can be safely conserved in liquid nitrogen at -196°C, through the process called cryopreservation. The feasibility study carried out by the group of independent Expert Group with support of the Task Force explores the need and potential operation mode of a safety back-up cryopreservation facility. The joint meeting of the Expert Group and the Task Force will discuss and make recommendations on the requirements for the facility and its operations, which will serve as necessary input to the final study report.

Objectives of the meeting

1. Analyse the results of the information gathering on cryo, field and *in vitro* genebanks and assess the potential need and requirements for a safety back-up cryopreservation facility.
2. Discuss and make recommendations on the principles, policies and procedures for the operation of the facility.
3. Discuss and make recommendations on the infrastructure, standards and costing of the facility.
4. Agree on the next steps in finalising the feasibility study and preparing the report.

DAY 1 (Monday, 15th of May)

Start	End	Topic
9:00	9:20	**Elena Popova:** Welcome and adoption of the agenda
		Session 1. Chaired by Alfredo Alves **State of conservation of vegetatively propagated and recalcitrant seed crops in genebanks**
9:20	10:00	**Elena Popova:** Presentation of the results of the survey on cryobanking and information gathering on field and *in vitro* collections • Survey results: current state and possible future expansion of cryopreserved collections in the 25 institutes surveyed • Field and *in vitro* collections around the world: the crops and number of accessions
10:00	10:30	Q & A, Discussion
10:30	10:45	TEA & COFFEE BREAK
		Session 2. Chaired by Dave Ellis **Needs for a cryopreservation facility, now and in the future**
10:45	12:30	Discussion: • Based on the information synthesis from Session 1, assess the requirements for the facility and its potential use Desired output: • Estimate the scale of potential use of the facility and list major potential users
12:30	13:30	LUNCH (provided in house)
		Session 3. Chaired by Stephen Adkins **Principles governing the facility's operation**
13:30	15:00	**Jane Toll:** Presentation of proposed principles for the facility's operation based on the model of the Svalbard Global Seed Vault • Technical and administrative requirements • Policy and governance requirements Q & A, Discussion **Michael Halewood** to join remotely
15:00	15:20	TEA & COFFEE BREAK
15:20	17:50	Continuation of Session 3 Desired output: Recommendations on: • Mandate and principles • Eligibility of collections • Obligations of users • Obligations of host • Governance/oversight mechanisms
18:30		Dinner (Brauhaus Boennsch, Sterntorbrücke 4, 53111 Bonn)

DAY 2 (Tuesday, 16th of May)

Start	End	Topic
9:00	9:20	**Elena Popova:** Agenda for the Day 2
		Session 4. Chaired by Jason Acker **Infrastructure, standards and costs of the facility**
9:20	10:20	Discussion introduction:w **Jason Acker**: Infrastructure, standards and costs based on medical repositories **Bart Panis, Steve Adkins**: Inputs from the PGRFA experience **Daniela Horna**: Costing the establishment and operation of the facility
10:20	10:35	Q & A, Discussion
10:35	10:50	TEA & COFFEE BREAK
10:50	12:30	Discussion on Session 4 Desired output: Recommendations on • Options for the location of facility • Management and technical standards and how they can be developed and agreed • Cost criteria and what cost-estimations are needed and how they can be done
12:30	13:30	LUNCH (provided in house)
		Session 5. Chaired by Bart Panis **Guidelines for depositors**
13:30	14:30	**Dave Ellis**: proposals for the guidelines • Requirements for sample replicates • Requirements for health and viability of the samples • Packaging and shipping requirements • Documentation requirements Desired output: • Recommendations on Depositor guidelines and how they can be developed and agreed
		Session 6. Chaired by Nicolas Roux **Addressing the challenges of cryopreservation**
14:30	14:50	**Elena Popova**: Status of crop cryopreservation and protocol development based on survey and data collection **Alfredo Alves**: Capacity issues facing national programs
14:50	15:10	TEA & COFFEE BREAK
15:10	16:00	Discussion • What is needed to develop operational protocols for other crops and address capacity building?
		Session 7. Chaired by Jane Toll **Conclusions and next steps**
16:00	17:30	Discussion • Main decisions points - recommendations • Gaps and pending work • Report outline Desired output: • Agreement on structure and content of the report and its preparation
17:30	18:00	**Elena Popova**: Meeting wrap-up and next steps
18:00		Departure of the participants

ANNEX 4: QUOTATIONS RECEIVED FROM COMMERCIAL CRYOBANKING FACILITIES: "BIOKRYO", GERMANY, "FISHER CLINICAL SERVICE", SWITZERLAND AND "CORE CRYOLAB", CANADA

BioKryo GmbH
Dr. Vincent v. Walcke-Wulffen
Managing Partner
Industriestraße 5
66280 Sulzbach/Saar

Telefon +49 (0) 6897/952 86 96
Telefax +49 (0) 6897/952 86 98 walcke@biokryo.de

Offer for the Storage under cryo-conditions for plant tissues of crops Global Crop Diversity Trust

BioKryo GmbH, a spin-off of the Fraunhofer-IBMT, stores biological samples of high value using a unique storage equipment with the new concept of Fraunhofer-IBMT for cryobanks, as they were introduced in the Fraunhofer-Bioarchive or the GHRC-HIV-Biobank. This concept illustrates an innovative standard for long-term storage of cryo- and bio-samples, as it is developed in the Fraunhofer-Cryostorage-Technology (FCT).

The BioKryo biobank stores human samples for therapeutic as well as for diagnostic purposes. BioKryo is certified for the storage of samples for therapeutic purposes according to §20c AMG (German Pharmaceuticals Act) as well as ISO 9001:2015 and AABB.

This quotation concerns the storage of plant tissues of crops under cryo-conditions using the standard cryostorage technology in a dedicated cryovessel. The samples have to be delivered prepared and frozen to the BioKryo biobank in Sulzbach.

Cryostorage in the vapor phase of liquid nitrogen

Standard cryostorage in a dedicated cryovessel

The modality of the standard cryostorage at BioKryo is based on the storage in a standard cryovessel at below -130°C in the vapor phase of liquid nitrogen. The samples will be stored in a dedicated cryovessel.

Monthly storage fee for one cryovessel of 420 litre volume for up to 200 cryoboxes for 81 or 100 vials of 2 ml exclusive VAT: 650,- € exclusive of VAT for BSL 2 category.

If a ten year contract is concluded the monthly price reduces to 590,-€ exclusive of VAT. If a second cryovessel is needed, a discount of 5% will be granted.

Storage fee

For each storage order, a fee of 15,- € exclusive VAT will be asked.

Removal from Storage

For each removal from storage, a fee of 15,- € exclusive VAT per sample or box will be asked. If a compilation of up to 10 vials is requested, a fee of 45,- € exclusive VAT will be asked. Shipping material, e.g. a Dry Shipper, has to be provided by the client. This material can be rent from BioKryo.

GMP documentation (optional)

For the provision of annual temperature data for the GMP-storage an annual fee of 250,-€

exclusive of VAT is sked.

Transport

Transport of one Dry Shipper

The transport of the samples is billed depending on the effort. For the organisation of the shipment, BioKryo asks a fee of 10 % of the carriers invoice amount.

Rental of Dry Shipper

The rental fee for one large IATA- MVE XC Dry Shipper for four cryoboxes is 160,- € exclusive VAT

The rental fee for one small IATA- QWick MiniMover Dry Shipper for 18 cryovials is 110,- € exclusive VAT

The rental is incl. a onetime conditioning with liquid nitrogen. The rental term is 10 days maximum. For each week after this period, a supplementary charge of 100,- € exclusive VAT for the large Dry Shipper and 75,- € exclusive VAT for the small Dry Shipper will be made.

Insurance of the Shipment (optional)

The shipment can be insured by Generali insurance. This insurance covers the case of total loss caused by accidents or thefts. The insurance premium is 5 ‰ of the value specified by the client.

Contract fee

For the completion of the contract is a one-time fee of 350,-€ exclusive of VAT is asked.

Cost Composition

Storage in a cryovessel	
Standard cryovessel 420 litre	650,- € p.m. plus VAT
GMP documentation (optional)	350,- € p.a. plus VAT
Storage fee	15,- € plus VAT
Removals	
Removal from storage of 1 cryovial or cryobox	15,- € plus VAT
Removal from storage of composition of 10 cryovials	45,- € plus VAT

Transport of up to four cryoboxes

Rent of a large Dry Shipper	160,- € plus VAT
Rent of a small Dry Shipper	110,- € plus VAT
Transport costs	to be clarified
Contract fee	350,- € plus VAT

Safety Installations of the BioKryo

BioKryo uses a Quality Management System, which is based on the "good practice" standard required by the German Pharmaceuticals Act (Arzneimittelgesetz, AMG). This QM-System is GMP conform. Since 2013, BioKryo is certificated after ISO 9001:2015. In 2016 BioKryo has been certified according to the AABB-Standard for blood banks.

An official operation authorization for the storage of therapeutic samples according to § 20c AMG (German Pharmaceuticals Act) had been issued for the BioKryo GmbH. Additionally, the storage premise has been declared as a laboratory of the biosafety level 2 (BSL2).

Each cryotank is equipped with a redundant full-automated surveillance system for temperature regulation and filling of liquid nitrogen. In case of emergency, all cryovessel keep the temperature below -130°C for 14 days. If after this period, the automated supply is still out of order, manual filling of liquid nitrogen is possible. Data of this surveillance system will be kept for the storage time of the samples and supplementary 30 years afterwards.

A redundant and full-automated surveillance system for the storage room including camera surveillance, several installations to measure the lack of oxygen, several ventilation systems, uninterrupted power supply as well as generators are installed.

Each cryotank is locked separately. The storage room is safeguarded with access control, alarm equipment and guard service.

Additional agreements

A storage contract will be drawn based on this offer, in which the modalities of storage will be described. The minimum contract term is five years.

The client engage himself to pay the declining balance of the cryovessel, if the client terminate the storage activity before contract term expire.

Time of Delivery

The storage services will start immediately after signing the contract.

Pecuniary loss insurance

On request, a consequential loss insurance component may be included in the storage contract, which is connected with additional costs. The basic liability is limited to the triple layer of rent money.

Costs

All prices are exclusive VAT, customs and shipping. Additional expenses such as taxes, customs and costs for shipment shall be borne by the customer. These expenses will be additionally charged to the customer. If, in agreement with the customer, travelling should be necessary, all travel expenses will be charged separately to the customer.

Expiration date

This quotation is valid until 31.10.2017. Thereafter it is subject to be changed.

Sulzbach, 15st June 2017

Vincent von Walcke-Wulffen

Fisher Clinical Services GmbH
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CH-4123 Allschwil
Switzerland
Telefon +41(0)61 485 23 00

Global Crop Diversity Trust
Julia Daniela Horna
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GERMANY
+49 176 568 457 48
jdhorna@fastmail.fm

June 20, 2017, VW

Quote

Customer Ref.: N/A
Protocol No.: Plant tissues LN2 storage
FCS #: 213473
Order 1, Version 1

Dear Daniela,

Thank you for the opportunity to provide pricing for services in regards to the above referenced project.

Fisher Clinical Services

Estimated Cost Summary

Activity	Cost
Clinical Ancillary Management Services	EUR 60.211,00
Estimated Total	**EUR 60.211,00**

Only services utilized will be billed.

The estimated costs are based on the assumptions listed in this Quote. If changes occur, pricing may increase or decrease. The above Estimated Cost Summary is intended for estimating purposes only and may or may not reflect actual total costs.

If the pricing contained in this Quote is based on a currency other than the functional currency of the Fisher Clinical Services entity named on the first page and the quoted currency fluctuates, Fisher Clinical Services may adjust the pricing based on the current foreign exchange ("FX") rate at the time of generating the invoices. If the invoice is not paid by the due date and additional FX rate changes occur, Fisher Clinical Services will issue an additional invoice to recover FX losses during the late payment period.

Version History

Version Number	Date	Description of Change	Previous Quote Value	Change in Value	New Quote Value
1	20/Jun/17	Created.			EUR 60.211,00

Fisher Clinical Services

Glossary

AOR	Acknowledgment of receipt
API	Active Pharmaceutical Ingredient
DEA	Drug Enforcement Agency
EU	European Union
FCS	Fisher Clinical Services
GMP	Good Manufacturing Practice
IRT	Interactive Response Technology
PO	Purchase Order
QA	Quality Assurance
QP	Qualified Person

Study Specifications

Project Scope	-5-6 Reception of 200 cryoboxes in 1 year -15 Receptions of 200 cryoboxes more in next 5 years -LN2 storage of plant tissues in a shared unit -Retrieval & Shipment preparation

Fisher Clinical Services

Pricing

BioServices

		Qty	Unit	Total	
01	**Set-Up Fees**			**1.600,00**	**EUR**
1.1	Set-Up Fees, including system set-up and preparation of project documentation	1	1.600,00	1.600,00	EUR
02	**Project Management Fees**			**12.600,00**	**EUR**
2.1	Monthly Project Management Fee,assuming 6 years project duration (if the project lasts longer the quote will be renewed after 6 years)	72	175,00	12.600,00	EUR
03	**Reception Materials / Samples**			**4.503,00**	**EUR**
3.1	Reception Fees, including QC check, assuming 25 receptions each of 15-40 cryoboxes	25	75,00	1.875,00	EUR
3.2	Inventory Fees per cryobox, assuming 420 cryoboxes in total and each box with an individual ID number	420	0,90	378,00	EUR
3.3	*in case every vial has an individual ID number and needs to be entered into the system, the inventory fees per vial will be charged - 0.90 €				
3.4	Upload of the inventory list into the electronic system, assuming 25 receptions	25	90,00	2.250,00	EUR
04	**Bio Sample Storage**			**38.880,00**	**EUR**
4.1	Monthly Storage Fees for plant tissues in a shared LN2 Tank, assuming 2 sections of a LN2 unit over 6 years duration of the project	72	540,00	38.880,00	EUR
05	**Retrieval & Special Handling**			**378,00**	**EUR**
5.1	Retrieval Fees per cryobox, assuming 420 boxes	420	0,90	378,00	EUR
06	**Distribution Fees**			**2.250,00**	**EUR**
6.1	Shipment preparation Fees, assuming 25 shipments of 15-40 boxes	25	90,00	2.250,00	EUR
07	**Freight Charges Estimate**				**EUR**
7.1	Estimated Freight Charges Fees for LN2 shipment optional and will be defined on request				
7.2	Dry shipper rental per dry shipper, including 1 data logger for temperature control		800,00		
08	**Extra Work**				**EUR**
8.1	Extra Work Fee in case of any not planned activities		120,00		
	Estimated Total costs BioServices			60.211,00	EUR

Fisher Clinical Services

Validity:
This Quote remains open for acceptance for 90 days after the date of the Quote.

Import Duties & VAT:
Our prices exclude import duties and VAT. Import duties and VAT will be charged at prevailing rates on our invoices.

The Fisher Clinical Services entity named on the first page of this Quote ("Fisher") hereby offers to provide the services set forth in this Quote (the "Services"), such offer expressly conditioned on the following: (i) a written master services agreement that governs the provision of services by Fisher is executed by both an authorized representative of Customer and an authorized representative of Fisher ("Master Services Agreement"); (ii) the Master Services Agreement is current and in effect as of the date of the performance of the Services by Fisher; and (iii) the sourcing of components and/or clinical ancillary materials set forth in this Quote ("Sourcing Services") will be performed in accordance with the Sourcing Terms and Conditions attached to this Quote and incorporated herein. By accepting this Quote, Customer agrees to accept and be legally bound by the terms and conditions stated in this Quote, and the terms and conditions stated in the Master Services Agreement with regard to the Services and the Sourcing Terms and Conditions with regard to the Sourcing Services. Customer may accept this Quote/offer by signing this Quote or providing Fisher with a Customer approved purchase order citing the quotation number stated on this Quote. Upon acceptance by Customer, this Quote shall serve as a Statement of Work (Individual Project Agreement or Work Order, as applicable) under the Master Services Agreement with regard to the performance of Services and the Sourcing Services. This Quote together with the Master Services Agreement and the Sourcing Terms and Conditions is the complete and sole statement of the agreement between Fisher and Customer with respect to the Services and the Sourcing Services; and any other terms and conditions, whether oral or written (including those terms and conditions stated in a document issued by Customer, including any Customer approved purchase order), are hereby expressly rejected by Fisher, and deemed null and void. To the extent that any terms or provisions of the Master Services Agreement conflict with the Sourcing Terms and Conditions, Customer and Fisher expressly agree that the Sourcing Terms and Conditions shall control for any and all Sourcing Services.

No waiver, consent, modification, amendment or change of the terms and conditions contained in this Quote or the Master Services Agreement or the Sourcing Terms and Conditions shall be binding unless subsequently agreed to in writing signed by authorized representatives of both Fisher and Customer. Fisher's failure to object to terms contained in any subsequent communication from Customer will not be a waiver or modification of the terms and conditions set forth in this Quote or in the Master Services Agreement or the Sourcing Terms and Conditions.

We hope our Quote meets your expectations. We would be delighted to support your project with our services and are looking forward to your feedback. Please do not hesitate to contact us at your best convenience for further information regarding our Quote.

With kind regards,
Fisher Clinical Services GmbH

V. Weinstein

Viktoria Weinstein
Program Manager

Mathieu Villarmé
Director Bioservices

Fisher Clinical Services

Acceptance

Customer, by signing below, agrees to the Pricing and Scope of this Quote and the Sourcing Terms and Conditions. Customer further understands that if there are changes to the specifications listed above, a revised Quote will be required.

Date:	
Name:	
Title:	
Signature:	
Legal entity to be invoiced:	
Customer Purchase Order #:	

Fisher Clinical Services

SOURCING TERMS AND CONDITIONS

UNLESS OTHERWISE EXPRESSLY AGREED IN WRITING, ALL SOURCING SERVICES ARE SUBJECT TO THE FOLLOWING TERMS AND CONDITIONS:

1. GENERAL. The Fisher Clinical Services entity named in the Quote ("**Seller**") hereby offers to (i) source the products listed in the Quote (the "**Products**") for the buyer named in the Quote ("**Buyer**"), (ii) order and take delivery of the Products; and (iii) verify quantities of Products received and carry out a standard inspection of the delivered Products in accordance with Seller's standard operating procedures (collectively, the "**Sourcing Services**") on the express condition that Buyer agrees to accept and be bound by the terms and conditions set forth in the Quote.

2. PRICE. All prices are conditioned upon Buyer purchasing the total quantities of the Product and taking delivery of it in accordance with the Quote. All prices are subject to adjustment due to changes in the specifications (including dosage or formulation), quantities, handling requirements, shipment arrangements or other assumptions in the Quote and/or the inclusion of other terms or conditions that are not set forth in the Quote.

3. DELIVERY; CANCELLATION OR CHANGES BY BUYER. The Products will be shipped to the destination specified by Buyer, EXW Seller's facility, unless otherwise agreed to in the Quote. The obligations of Seller to perform the Sourcing Services under the Quote are conditioned upon Seller's supplier providing the required quantity of the Products. Seller will have the right, at its election, to make partial shipments of the Products and to invoice each shipment separately. Seller reserves the right to stop delivery of Products in transit and to withhold shipments in whole or in part if Buyer fails to make any payment to Seller when due or otherwise fails to perform its obligations hereunder. All shipping dates are approximate only, and Seller will not be liable for any loss or damage resulting from any delay in delivery or failure to deliver which is due to any cause beyond Seller's reasonable control. In the event of a delay due to any cause beyond Seller's reasonable control, Seller reserves the right to terminate the order or to reschedule the shipment within a reasonable period of time, and Buyer will not be entitled to refuse delivery or otherwise be relieved of any obligations as the result of such delay. Products as to which delivery is delayed due to any cause within Buyer's control may be placed in storage by Seller at Buyer's cost, risk and expense and for Buyer's account. Orders in process may be canceled only with Seller's written consent and, in addition to all other amounts owed to Seller for Sourcing Services rendered, upon payment of Seller's cancellation charges equal to five percent (5%) of the total value of the Quote, which amount represents a reasonable estimate of Seller's costs related to such cancellation and not a penalty of any kind. Orders in process may not be changed except with Seller's written consent.

4. TITLE AND RISK OF LOSS. Notwithstanding the trade terms indicated above and subject to Seller's right to stop delivery of Products in transit, title to and risk of loss of the Products will pass to Buyer upon the successful completion of the verification and inspection services set forth in Section 1(iii) above. The expense and risk of loss and/or damage for deliveries and shipments of the Products by Seller will be borne by Buyer, notwithstanding the use of any delivery term on any waybill, import/export forms or other documentation relating to such delivery and/or shipment. Seller expressly disclaims any and all liability for the actions or omissions of all third parties, including, without limitation all storage providers, delivery services and carriers.

5. WARRANTY DISCLAIMER. Products supplied by Seller that are obtained by Seller from an original manufacturer or third party supplier are not warranted by Seller, but Seller agrees to assign to Buyer any warranty rights in such Product that Seller may have from the original manufacturer or third party supplier, to the extent such assignment is allowed by such original manufacturer or third party supplier. TO THE FULLEST EXTEXT ALLOWED BY LAW, SELLER EXPRESSLY DISCLAIMS ANY AND ALL WARRANTIES, WHETHER EXPRESS OR IMPLIED, ORAL OR WRITTEN, WITH RESPECT TO THE PRODUCTS OR OTHERWISE ARISING OUT OF THE SOURCING SERVICES, THE PRODUCTS AND THIS QUOTE, INCLUDING WITHOUT LIMITATION ALL IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

6. INDEMNIFICATION. Buyer shall indemnify, defend with competent and experienced counsel and hold harmless Seller, its parent, subsidiaries, affiliates and divisions, and their respective officers, directors, shareholders and employees, from and against any and all damages, liabilities, actions, causes of action, suits, claims, demands, losses, costs and expenses (including without limitation reasonable attorneys' fees and disbursements and court costs) to the extent arising from or in connection with (i) Buyer's purchase, ownership, further distribution or use of the Product. (ii) Seller's compliance with designs, specifications or instructions supplied to Seller by Buyer; (iii) use of a Product in an application or environment for which it was not designed and/or in violation of the Quote; or (iv) modifications of a Product by Buyer or person or entity acting on behalf of Buyer.

7. LIMITATION OF LIABILITY. TO THE FULLEST EXTEXT ALLOWED BY LAW, NOTWITHSTANDING ANYTHING TO THE CONTRARY CONTAINED HEREIN, THE LIABILITY OF SELLER UNDER THESE SOURCING TERMS AND CONDITIONS (WHETHER BY REASON OF BREACH OF CONTRACT, TORT, INDEMNIFICATION, OR OTHERWISE) SHALL NOT EXCEED THE LESSER OF (i) US $100,000, or (ii) THE TOTAL PURCHASE PRICE THERETOFORE PAID BY BUYER TO SELLER WITH RESPECT TO THE PRODUCT(S) GIVING RISE TO SUCH LIABILITY. ADDITIONALLY, NOTWITHSTANDING ANYTHING TO THE CONTRARY CONTAINED HEREIN, IN NO EVENT SHALL SELLER BE LIABLE FOR ANY INDIRECT, SPECIAL, CONSEQUENTIAL, INCIDENTAL, PUNITIVE OR EXEMPLARY DAMAGES (INCLUDING WITHOUT LIMITATION DAMAGES FOR LOSS OF USE OF FACILITIES OR EQUIPMENT, LOSS OF REVENUE, LOSS OF DATA, LOSS OF PROFITS OR LOSS OF GOODWILL), REGARDLESS OF WHETHER SELLER (a) HAS BEEN INFORMED OF THE POSSIBILITY OF SUCH DAMAGES OR (b) IS NEGLIGENT.

8. EXPORT RESTRICTIONS. Buyer acknowledges that each Product, including technical information supplied by Seller or

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contained in documents (collectively, "**Items**"), may be subject to export controls of the U.S. government and/or other governmental authorities. The export controls may include, but are not limited to, those of the Export Administration Regulations of the U.S. Department of Commerce, which may restrict or require licenses for the export of Items from the United States and their re-export from other countries. Buyer shall comply with all applicable laws, regulations, laws, treaties, and agreements relating to the export, re-export, and import of any Item. Buyer shall not, without first obtaining the required license to do so from the appropriate government agency: (i) export or re-export any Item, or (ii) export, re-export, distribute or supply any Item to any restricted or embargoed country or to a person or entity whose privilege to participate in exports has been denied or restricted by the U.S. government or other applicable governmental authority. Buyer shall cooperate fully with Seller in any official or unofficial audit or inspection related to applicable export or import control laws or regulations, and shall indemnify and hold Seller harmless from, or in connection with, any violation of this Section by Buyer or its employees, consultants, agents, or customers.

9. MISCELLANEOUS. (a) In the event of any legal proceeding between the Seller and Buyer relating to this Quote, neither party may claim the right to a trial by jury, and both parties waive any right they may have under applicable law or otherwise to a right to a trial by jury. Any action arising under this Quote must be brought within one (1) year from the date that the cause of action arose. (b) The application to this Quote of the U.N. Convention on Contracts for the International Sale of Goods is hereby expressly excluded. (c) In the event that any one or more provisions contained herein shall be held by a court of competent jurisdiction to be invalid, illegal or unenforceable in any respect, the validity, legality and enforceability of the remaining provisions contained herein shall remain in full force and effect, unless the revision materially changes the bargain. (d) Seller's failure to enforce, or Seller's waiver of a breach of, any provision contained herein shall not constitute a waiver of any other breach or of such provision. (e) Unless otherwise expressly stated in the Quote, the Products shall be used for clinical trials only and shall not to be used for resale, commercial trade or any other purpose. (f) Buyer agrees to cooperate fully and execute any and all documents, including any certifications or other documents required by Seller's supplier relating to the use of the Products, and to take all additional actions which may be necessary or appropriate to give full force and effect to the terms and conditions set forth herein.

CRYOLAB
for the future

July 5, 2017

Daniela Horna
Email: jdhorna@fastmail.fm

Storage Quotation
Quotation Reference Number: 070520171

Thank you for reaching out to us for a storage quote. We are pleased to provide you with the following cryo-storage quotation for your consideration.

Your storage requirements include:

Storage of 200-400 boxes (81 cell boxes) of samples
Plant meristematic cells

No samples transferred to Core are infectious
Minimal withdrawals with periodic deposits

About Core Cryolab

For 12 years Core Cryolab has been providing world-class service to some of North America's most prestigious principle investigators, research institutes, clinical studies and biopharmaceutical communities.

Core is uniquely positioned as an authorized distributor of Chart MVE cryogenic equipment and an end user of the products we sell and service.

Our experienced staff, redundant security systems and robust quality system ensure that samples are safely stored and maintained

Superior Quality System – as part of our agreement with multiple clients our facility has been inspected by accrediting and regulatory bodies including: AABB, Netcord-FACT, and Health Canada.

Centrally located at Toronto General Hospital ensuring we are in a priority facility for emergency response in the event of emergency.

Storage cost overview for dedicated Chart MVE 1542R Freezer: $2,040/month*

Freezer capacity is 364 x 81 cell boxes and 224 x 25 cell boxes (equivalent of total of >400 boxes of samples)

To receive this price a minimum 5 year contract would be required.

*Pricing is in Canadian Dollars.

Services included in the storage fee:

Samples will be stored in validated freezer. Freezer connected to central supply system and includes duel redundant monitoring

- Monitor will be connected to hospital emergency power
- 2 - 3000L LN2 supply tank delivered through vacuum jacketed pipe o Security System, both surveillance and alarm monitoring

Receipt and Accession of Customer Samples

- Cross reference system
- Deposit / Withdrawal records would be issued for all transactional activity

Sample Pick up / Delivery Services

- Samples can be shipped using Core Cryolab validated shippers

24-7-365 Monitoring

- Hospital Security Department monitors cameras and alarms
- Core Cryolab staff on-call after hours to respond to any alarms

Freezer Maintenance

- Weekly physical liquid level measurement
- Monthly, Quarterly testing and maintenance
- Preventative Maintenance Program according to manufacturer's recommendations

Record Keeping

- Temperature Logs
- Equipment Validation
- Equipment Maintenance
- Inventory Management
- Tracking and Labeling mechanism

Emergency Preparedness Plans in Place

Sample Deposits / Withdrawals

Core Cryolab operates a large fleet of Chart MVE Cryogenic Dry LN2 Vapor shippers. Our shippers have varying capacities and we can easily transfer anywhere from one vial to 308 boxes in a single shipment.

Request 2 business day notification of transactional activity to prepare our vapour shippers for deposits or withdrawals.

Thank you for the opportunity to provide cryostorage services. We look forward to continued discussions.

Regards,

Patrick Storr
Core Cryolab
Direct: 416-260-2673
Toll Free: 1-866-580-9872

ANNEX 5: LIST OF ACRONYMS

Bioversity	Bioversity International
BPI	Bureau of Plant Industry
CAAS	Chinese Academy of Agricultural Sciences
CARBAP	Centre Africain de Recherche sur Bananiers et Plantains
CATIE	Centro Agronómico Tropical de Investigación y Enseñanza
CCSM-IASP	Centro de Citricultura «Sylvio Moreira», Instituto Agronomico de São Paulo
CENARGEN	Embrapa Genetic Resources and Biotechnology
CePact	Centre for Pacific Crops and Trees
CGIAR	Consultative Group of International Agriculture Research
CIAT	International Center for Tropical Agriculture
CIP	International Potato Centre
CNR	National Research Council
CNRG/INIFAP	Centro Nacional de Recursos Genéticos, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias
CPCRI	Central Plantation Crops Research Institute
CRI	Crop Research Institute
Crop Trust	Global Crop Diversity Trust
CRU/UW	Cocoa Research Unit, University of West Indies
CULS	Czech University of Life Sciences Prague, Czech Republic
DV	Droplet Vitrification
ECICC	Estación Central de Investigaciones de Café y Cacao
ED	Encapsulation Dehydration
EMBRAPA	Brazilian Agricultural Research Corporation
FAO	Food & Agriculture Organization of the United Nations
FAOSTAT	Food & Agriculture Organization of the United Nations Statistics
GEN	Plant Genetic Resources Unit, Cornell University, New York State Agricultural Experiment Station, United States Department of Agriculture, Agricultural Research Services
GRC NARO	Genetic Resources Center, National Agriculture and Food Research Organization
HRI	Hop Research Institute, Zatec, Czech Republic
IAC	Instituto Agronômico de Campinas
ICAR	Indian Council of Agricultural Research
ICQC-R	UK University of Reading's International Cocoa Quarantine Centre
ICRAF	World Agroforestry Centre
IFVC	Institute for Field and Vegetable Crops
IITA	International Institute for Tropical Agriculture
InHort	Research Institute of Horticulture
INIAP	National Institute for Agricultural Research
INTA	Instituto Nacional de Tecnología Agropecuaria

IPK	Leibniz Institute of Plant Genetics and Crop Plant Research
IPRI	Indonesian Palmae Research Institute
IRD	Institute of Research for Development
ISBER	International Society for Biological and Environment Repositories
JARC	Jimma Agricultural Research Centre
JKI	Julius Kühn-Institut
KU Leuven	Katholieke Universiteit, Leuven
LN	Liquid nitrogen
Luke	Natural Resources Institute Finland
MARDI	Malaysian Agricultural Research and Development Institute
MCB	Malaysian *Cocoa* Board
MHRP	Main Highlands Research Programme
MSBKew	Millenium Seed Bank
MU	Mendel University, Brno
NARO	National Agriculture and Food Research Organization
NBPGR	National Bureau for Plant Genetic Resources
NGRC	Nordic Genetic Resource Centre
NLGRP	National Laboratory for Genetic Resources Preservation
NIAS	National Institute of Agrobiological Sciences
NTBG	National Tropical Botanical Gardens
PCA	Philippine Coconut Authority
PGRC	Plant Gene Resources of Canada
PGRRI	Plant Genetic Resources Research Institute
Plant Treaty	International Treaty on Plant Genetic Resources for Food and Agriculture
PRI	Potato Research Institute, Havlickuv Brod, Czech Republic
RBIP	Research and Breeding Institute of Pomology, Holovousy, Czech Republic
RDA	Rural Development Administration
SAARI	Serere Agriculture and Animal Production Research Institute
Seed Vault, or SGSV	Svalbard Global Seed Vault
SOPs	Standard Operating Procedures
SPC	South Pacific Community
UAC	Université d'Abomey-Calavi
UNCI	Université Nouvelle de Côte D'Ivoire
USDA	United States Department of Agriculture
VIR	N. I. Vavilov Institute of Plant Genetic Resources
WLMP	Sir Alkan Tololo Research Centre, Bubia

ANNEX 6: REFERENCES

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Appendices section photo credits:
In vitro banana accessions conserved by Bioversity International at the International Transit Centre, Leuven, Belgium.
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Annexes section photo credits:
Excision of the banana meristem tip that will be cryopreserved.
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