Bioversity International

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Is cryopreservation a viable method for long-term conservation of coffee biodiversity?

IMPACT ASSESSMENT BRIEF NUMBER 6

Bioversity International's series of Impact Assessment Briefs aims to inform readers about the major results of evaluations carried out by the centre. The Briefs summarize conclusions and methods of more formal papers published in peer-reviewed journals.

Coffee is not only one of the most popular beverages in the world, but coffee beans are also the world's most valuable agricultural export commodity, with an export value of US$6.2 billion in 2007. Most coffee is produced in the developing world by small-scale farmers, many of whom are resource poor and depend on coffee trade for their livelihoods.

The success of the crop—and of the whole coffee industry built upon it—depends on the availability of diversity to enhance the genetic base of coffee and provide resistance to pests and diseases. Coffee comes from three main cultivated species of the genus *Coffea*: *Coffea arabica*, *C. canephora* and *C. liberica*. The forests of West and Central Africa, south-western Ethiopia and neighbouring countries are the centres of origin of cultivated *Coffea* species. Aside from farmers growing traditional coffee varieties in their fields, these forests are the ultimate sources of coffee genetic diversity. However, deforestation, encroachment of agricultural activities, population pressures and economic hardship are threatening these natural resources. There is thus an urgent and continuing need to collect and conserve coffee genetic resources.

E. Dulloo/Bioversity International

Unfortunately, coffee cannot be conserved as seed using conventional methods—i.e. drying and storage at low temperature. As a result coffee germplasm has traditionally been conserved in field genebanks—plants grown in the open. But field genebanks have a number of disadvantages, including the limited extent of genetic diversity that can be conserved in them, the high risk of loss from pests and diseases, and their vulnerability to weather and other external risks such as fire. They also have high maintenance costs and require a lot of space, time and labour.

In a field genebank, the accessions are grown out in the same field, making them particularly susceptible to pests and diseases. This vulnerability is accentuated by the fact that many accessions may not be adapted to the local environments of the field genebank. Local climate and other environmental conditions represent strong selection pressures on individuals in field genebanks and contribute to skewed genetic erosion in field collections.

To address this problem, researchers at the Institut de Recherche pour le Développement (IRD), France, developed and optimized a cryopreservation protocol for coffee seeds. In 1999 and 2000, it was transferred to the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica, with support from Bioversity International. CATIE manages one of the largest field collections of *Coffea arabica* in the world: 1,992 accessions, which are maintained as live plants on approximately 9 hectares of land.

The new cryopreservation protocol was first applied to a subset of the CATIE coffee collection comprising 63 genetically distinct coffee varieties (genotypes), creating the first coffee cryo-bank in the world. This provided the ideal case to investigate the question as to whether these new techniques are more cost effective than field collections and more efficient at reducing genetic erosion, allowing the costs of cryopreservation and field collection to be compared at the same location.

No systematic data are available on loss of coffee diversity from field genebanks. However, losses have been estimated at 5 to 10% of accessions annually in banana, another crop commonly conserved in field genebanks, and there are cases of entire collections being lost to a single event, such as the coffee collection at Ilaka Est, Madagascar, which was destroyed by a cyclone.

A study carried out by Bioversity International and its partners in 2008 investigated two major issues relating to conservation of coffee germplasm:

1. What are the real threats to and losses of coffee germplasm held globally in field genebanks?
2. How do the costs of cryopreservation compare with those of maintaining coffee field collections for long-term conservation?

This brief reports on the findings of the 2008 study.

Study methods

Data were collected on the collection at CATIE, and on other major coffee collections in Brazil, Ethiopia, Kenya and Madagascar. Questionnaires were used to gather information on the number of accessions conserved over time, the numbers of accessions lost over time and number of individuals per accession. Data were also collected on the costs of field and cryopreservation, establishing the genebank and annual maintenance (including field preparation and plantation, health management, weeding, fertilizer application, irrigation, and labour). Data on the status of major coffee collections around the world were extracted from published literature and contact with the collection holders.

A detailed cost study was conducted on the field genebank and cryo-collection at CATIE, using data from CATIE and IRD. The study examined capital costs, quasi-fixed costs, variable costs, and in-perpetuity costs. Projections of costs were made for cryo-collections of 500, 1000 and 2000 accessions, in addition to the actual costs of the existing facilities (maximum capacity of 300 accessions).

Status of coffee germplasm in field genebanks

The extent of losses of accessions varied widely among the collections surveyed. CATIE has lost 125 out of a total 2,117 accessions since the collection's establishment (roughly 6%), while overall Madagascar has lost 46% of its accessions (146 out of 319 accessions). Brazil reported no losses of accessions, Kenya reported no losses since 1965, and Ethiopia has lost some 12.5% of the accessions in its collections.

The extent of losses is primarily related to the availability of funding for maintenance activities—such as weeding, irrigation, fertilizer application, pest and disease control, and shade management. The coffee collection in Brazil, for example, is well funded, whereas that in Madagascar suffered from lack of financial resources for maintenance. The budget allocated for collection maintenance at Kianjavato, Madagascar, was negligible and no maintenance work was performed for several years, leading to loss of accessions.

> **"Cryopreservation theoretically offers a more secure means of conserving germplasm than do field genebanks."**

Costs of cryopreservation versus field collections

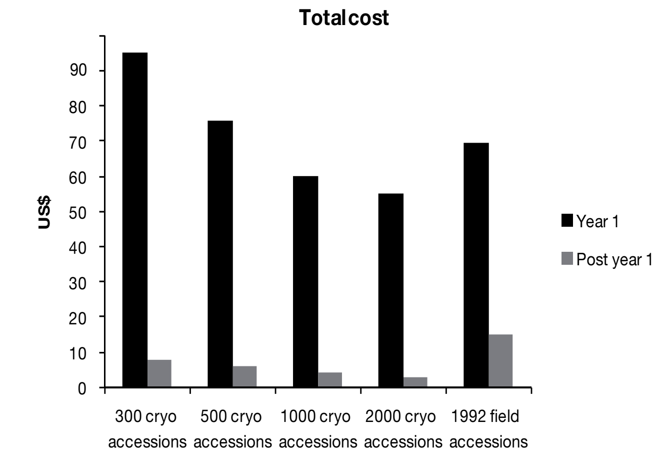
The comparison of costs for cryopreservation and maintenance of the field genebank at CATIE showed that the current cryo-collection, with 63 coffee accessions, is not cost effective—at US$95, the establishment cost per accession (calculated based on the 300 accession capacity of the current facilities) is a third more than the establishment cost per accession of the field collection (US$69.62), although the annual maintenance cost of cryopreservation is only half that of the field genebank (US$8 per accession for cryopreservation compared with US$15 per accession in the field genebank). However, if the size of the cryo-collection were increased to 2,000 accessions, the establishment cost per accession would fall to US$55 (21% less than that of the field collection), while the annual maintenance cost per accession would be US$3—one-fifth the US$15 cost for the field collection (Figure 1). The most significant cost increase (excluding labour costs) would be the capital cost of purchasing additional storage tanks for the liquid nitrogen.

These figures are in the same cost range (US$50–75) as that reported by others—such as the USDA for the establishment of a cryopreserved temperate fruit collection at Corvallis on the US west coast. Cryopreservation allows the exploitation of economies of scale—the more accessions there are in cryopreservation storage, the lower the per-accession cost.

In addition, cryo-collections do not require regular regeneration to maintain vigour of the conserved material, whereas material in field genebanks must be regularly regenerated to prevent loss of accessions due to senescence and other factors. Field genebanks thus need larger and more frequent investments to continue to function effectively, which makes them very vulnerable to losses if operating funds are unavailable for even one year. Where the collection holds rare or endangered germplasm, such losses could cause extinction.

Cryopreservation as a strategy for the future

Cryopreservation theoretically offers a more secure means of conserving germplasm than do field genebanks. Accessions in a cryo-collection are not at risk from unfavourable weather, vandalism, pests or diseases. Moreover, maintaining a field collection costs more than maintaining the same number of accessions in cryopreservation facilities. This makes field collections vulnerable to losses if financ-



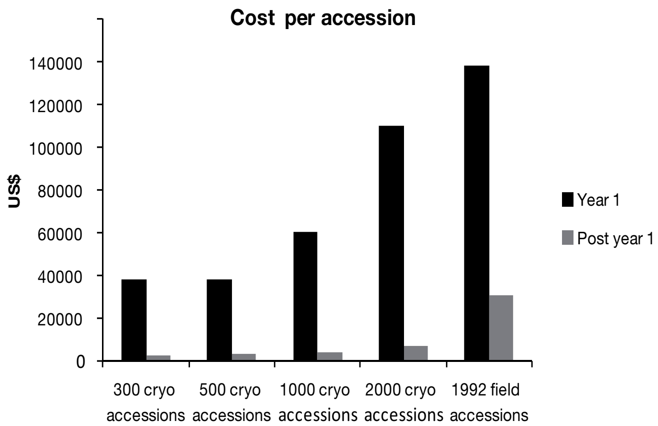


Figure 1. Cost of conserving coffee accessions (total and per accession) under cryopreservation and in a field genebank at CATIE, Costa Rica.

ing for annual maintenance is unavailable for one or more years. This can be especially serious when field collections hold rare or endangered germplasm and losses caused by sporadic and insufficient financing can thus lead to extinction. The lower annual maintenance cost of cryo-collections, along with the longer intervals between required capital expenditures, make cryopreservation less vulnerable to the funding cuts and inconsistencies that are a reality for many genebanks.

In addition to cost, cryopreservation has many other advantages over field genebanks. It is a clean, environmentally friendly technology that does not use any pesticides or fertilizers. Cryopreservation requires much less space than a field genebank. The conservation of 1,992 coffee accessions in the field at CATIE required 9 hectares of land while the same number of accessions could be cryopreserved within 10 square metres. The number of genotypes that a field genebank can hold is also restricted by human, financial and land resources, thereby limiting the genetic diversity it can conserve.

The cryopreservation protocol developed by IRD, and successfully tested in CATIE, allows cryopreserved seeds to be germinated normally following rewarming, eliminating the need for *in vitro* culture and hence reducing the need for highly skilled staff and expensive culture facilities.

"The costs of cryopreservation should not be considered prohibitive to establishing duplicate, back-up collections of coffee"

Cryo-banking of coffee seeds is already a reality: over 220 accessions of various coffee species are already safely duplicated in liquid nitrogen at IRD Montpellier. Most of these accessions come from the field collection maintained by IRD in La Réunion and from those of several countries in Latin America and the Caribbean.

Conclusions

This study shows that the costs of cryopreservation should not be considered prohibitive to establishing duplicate, back-up collections of coffee. If cryopreservation is adopted as a complement to field genebanks, the costs of cryopreservation must be added to existing costs of field conservation. However, field collections could be reduced and at least partly replaced with cryopreserved collections. Further studies are needed to establish the optimal balance between cryopreservation and field collection to ensure the long-term, cost-efficient conservation of coffee genetic resources.

Given the importance of genetic diversity to coffee producers—many of whom are small farmers depending on coffee for their livelihoods—policymakers from coffee-producing countries should lay the groundwork for establishing additional cryo-collections to ensure the future health of the crop. A regional or global cryopreserved collection could be established for coffee germplasm (as has been done for other crops such as banana) in which the costs of cryopreservation and the benefits derived from germplasm conservation could be shared among partner countries.

This study investigated coffee as a test case, but the results are relevant to other species that are difficult to conserve using the traditional method of seed drying and low-temperature storage. The underlying objective of the study was to assess the potential impact of the widespread adoption of cryopreservation for more species and in more genebanks.

This brief is based on Dulloo M.E., Ebert A.W., Dussert S., Gotor E., Astorga C., Vasquez N., Rakotomalala J.J., Rabemiafara A., Eira M., Bellachew B., Omondi C., Engelmann F., Anthony F., Watts J., Qamar Z. and Snook L. 2009. *Cost efficiency of cryopreservation as a long-term conservation method for coffee genetic resources*. Crop Science 49:2123–2138.

Citation: Gotor E., Cherfas J. 2011. *Is cryopreservation a viable method for longterm conservation of coffee biodiversity?* Bioversity International Series of Impact Assessment Briefs, no. 6. Bioversity International, 4 p.

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DECEMBER 2011

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