FIELD COFFEE COLLECTIONS AT RISK: CAN CRYOPRESERVATION HELP TO ENSURE THEIR LONG TERM SECURITY?

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1. INTRODUCTION[1]

While the world's consumers are happily enjoying their daily cups of coffee, coffee genetic resources, which form the basis for improved coffee production and quality, are rapidly becoming less diverse. Coffee is not only the source of one of the most popular beverages in the world, but it is also the world's most valuable agricultural export commodity, with a annual export value of US$9 billion (ICO, 2006). Most coffee-producing countries are in the developing world (ICO, 2006). Unlike other commodities, coffee is grown predominantly by small-scale farmers, most of whom are poor; the coffee trade is vital for their livelihoods.

The success of the coffee industry depends on the availability of a diversity of coffee germplasm to enhance the genetic base and provide resistance to pests and diseases such

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as coffee berry disease (*Colletotrichum kahawae*), coffee rust (*Hemileia vastatrix*) for *C. arabica*, and Fusarium wilt (*Fusarium xylarioides*) for *C. canephora*, as well as bacterial blight of coffee (*Pseudomonas syringae*), root-knot nematodes (*Meloidogyne* spp.), and major insect pests of coffee such as coffee berry borer (*Hypothenemus hampei*) and the leaf miners (*Leucoptera* and *Perileucoptera* spp.). The sources of resistance to these pests and diseases, on which most breeding programmes have so far depended, still need to be sought and identified. Other benefits obtained from coffee genetic diversity include adaptation to abiotic stresses such as drought and enhancement of qualities such as aroma and flavour. For these reasons, genetic resources are of great value for breeding programmes.

Hein and Gatzweiler (2006) assessed the value of *Coffea arabica* genetic resources in the Ethiopian highlands for breeding programmes and obtained a net present value for coffee genetic resources of US$1.4 billion and US$420 million, at discounted rates of 5% and 10% respectively, based on a 30-year discounting period. Conserving these valuable resources is therefore critical for the future development and security of the crop. Because of the nature of coffee seeds, they cannot be conserved *ex situ* using traditional methods. This paper provides a brief overview of the collection and conservation options for coffee genetic resources, and assesses the potential of cryopreservation of coffee, as a complement to field collection, in terms of the cost efficiencies of these two conservation methods and the long-term viability and security of accessions.

2. BACKGROUND

Much of the diversity of coffee genetic resources is still found in the wild. The forests of West and Central Africa, south-western Ethiopia and neighbouring countries are the centres of origin of the cultivated species of *Coffea*. Together with farmers' fields growing traditional coffee varieties, these are the ultimate sources of coffee genetic diversity. The forests of Madagascar are the home of the relatively isolated Mascarocoffea section of the genus, characterized by low levels, or even absence, of caffeine. Deforestation and encroachment by agricultural activities, population pressures and economic hardships threaten all these reservoirs of genetic diversity. Their loss would represent significant erosion of the *Coffea* genepool (Gole *et al*., 2002).

Concern for this loss of coffee genetic diversity has stimulated a series of collecting missions by FAO, ORSTOM (now IRD), CIRAD and IBPGR (now Bioversity

International) (Fernie, 1968; Guillaumet and Hallé, 1978). Coffee, like many other important species of tropical origin, cannot be conserved as seed using traditional methods of drying and low- temperature storage because of its intermediate and recalcitrant seed-storage behaviour (Ellis et al., 1990; Hong and Ellis, 1995). Until the recent development of cryopreservation and other methods, coffee could only be conserved in field genebanks, *in situ* or on farm. Field coffee collections have been established in Ethiopia, Côte d'Ivoire, Cameroon, Kenya, and Madagascar (Berthaud and Charrier, 1988). Côte D'Ivoire also holds a collection of mainland African coffee species. Madagascar holds the world's largest collection of Mascarocoffee species, composed of 56 botanical species and 57 undescribed populations (Dulloo et al., 2001). Collections were also established in other regions of the world where coffee is an important commodity. In the Americas, coffee collections (mainly *Arabica*) were established in Costa Rica, Colombia and Brazil, while in Asia, Indonesia and India hold important collections (see Table 1). There are also a number of small collections of *Coffea* germplasm, intended mainly for short-term storage, in Portugal, France, Germany (Frison and Serwinski, 1995), Peru, Rwanda, Zaire and Hawaii (Bettencourt and Konopka, 1988). FAO (1998) estimates that 21,087 coffee accessions are conserved worldwide.

Field collections can provide major advantages for characterization, evaluation, identification and sometimes distributional purposes (Reed et al., 2004). However, field collection is far from being an optimal conservation method (Dulloo, 1998; 2001). After more than 50 years in existence, many coffee field collections are thought to be at risk as a result of several technical and economic factors, and the genetic diversity they maintain is being lost at a considerable rate. Various authors (Maxted et al., 1998; Dulloo et al., 1998; Engelmann and Engels, 2002, Reed et al., 2004) have underlined the disadvantages of field collections, particularly the limited extent of genetic diversity that they can conserve; the high risk of genetic erosion due to pest and diseases; their vulnerability to weather and other external risks such as fire, vandalism and policy changes; and their high maintenance costs, space and time requirements, and labour intensiveness. An additional constraint to the exchange of germplasm obtained through the transfer of vegetative material from field genebanks is the risk of disease transfer.

These problems are not unique to coffee, applying equally to other species held in field genebanks such as banana, coconut, tropical fruit trees, cacao and other vegetatively

propagated species. The fact that field collections are at risk of heavy losses of diversity is of major concern. Swennen and Panis (pers. com.) report that 5-10% of accessions in *Musa* field collections are lost each year. Togo lost its entire yam field collection due to a fire in 2000 (Vodouhe, pers. com).

Bioversity International, the world largest institute concerned with the conservation of agricultural biodiversity, has long recognized the problem of *ex-situ* conservation of crops that cannot be conserved using traditional methods, and has been investing its efforts in developing novel conservation technologies for these species. Research in novel conservation technologies has accelerated with the development of biotechnology. *In-vitro* slow growth conservation methods have often been cited as good ways of complementing and providing backup to field collections (Engelmann, 1991; Dulloo et al., 1998). For coffee, an in-vitro slow growth method has been developed for short-term conservation (Dussert 1997a) and an in-vitro coffee core collection, genetically representative of the large field genebank maintained in Côte d'Ivoire, was set up at ORSTOM (now IRD). However, Dussert (1997b) showed that while some genetic groups were well adapted to in-vitro conditions and could therefore be maintained through the three-year duration of the experiment, other genetic groups could not be conserved in-vitro and were lost rapidly. This resulted in genetic drift of the *in-vitro* coffee core collection, demonstrating that in-vitro conservation is not a viable option for the conservation of coffee genetic resources.

Pollen conservation is another possible conservation method, but its application is limited (Dulloo et al., 1998). The development of cryopreservation provides a more promising option (Engelmann and Engels, 2002). In cryopreservation, living tissues are conserved at very low temperatures – usually at -196ºC – in liquid nitrogen, to arrest mitotic and metabolic activities and guarantee the long-term preservation of germplasm in a genetically unaltered state. The plant material can be stored without alteration or modification for a theoretically unlimited period of time. In addition, cultures are stored in small volume, protected from contamination, and require very limited maintenance (Engelmann, 1997).

Research on cryopreservation has led to the development of protocols for cryopreservation of over 200 different plant species including *Musa*, coffee and citrus (Takagi and Engelmann, 2000; Engelmann, 2004). For *Coffea arabica*, which is autogamous and seed-propagated, considerable efforts were made at IRD-Montpellier (France) to

investigate why coffee seeds were sensitive to liquid nitrogen (LN) storage. This led to the development of a successful cryopreservation procedure, which was applied to a core subset of the CATIE coffee collection (Vásquez et al., 2005; Dussert et al., 2007), providing a viable alternative for conserving coffee genetic resources.

According to the Convention on Biological Diversity (CBD), countries have the obligation to take measures to ensure both in-situ and ex-situ conservation of the genetic resources under their sovereignty (Articles 8 and 9) (UNCED, 1992). Other global initiatives such as the Global Strategy for Plant Conservation and the International Treaty on Plant Genetic Resources for Food and Agriculture have been developed with the aims of halting the current and continuing loss of plant diversity and facilitating multilateral access. These strategies recommend the establishment of complementary *ex-situ* measures in countries of origin. Since the original diversity of coffee germplasm is in Africa, African countries have the principal burden of ensuring long-term conservation. Alternative or complementary *ex-situ* conservation technologies must be suitable to the African context by being low cost (or at least not cost-prohibitive as compared to other methods) and easily implemented with the technological capacity available in African genebanks.

Bioversity International carried out a survey in November 2006 of genebank managers and specialists in cryopreservation to assess their perspectives on potential impacts of cryopreservation and obstacles to its more widespread adoption (see Table 10). Most respondents reported that the greatest potential impacts of cryopreservation are:

- reducing losses of genetic diversity from diseases, insect attacks, storms and natural disasters that threaten field collections;
- ensuring greater genetic integrity since accessions are not contaminated by germplasm from neighbouring plants; and
- facilitating the use of germplasm since accessions are more easily replicated and transferred as in-vitro materials.

When queried about obstacles to the more widespread adoption of cryopreservation, however, respondents identified lack of trained personnel, lack of protocols and establishment and maintenance costs.

Although the survey results indicate that specialists believe cryopreservation to be expensive, few studies have analysed the actual costs and effectiveness of cryopreservation or compared these costs to the costs of field genebanks. Hummer and Reed (1999) calculated the cost of establishing a cryo-collection of temperate fruit trees maintained at the USDA-ARS National Clonal Germplasm Repository in Corvallis, Oregon, USA at US$50 to US$75 per accession (not including labour costs) and approximately $US1 for annual upkeep per accession. Several authors (Jarret and Florkowski, 1990; Epperson et al., 1997; Pardey et. al., 1999, 2001; Saxena et al., 2002; Virchow, 1999, 2003; Burstin et al.,1997) have documented the conservation costs of a wide range of crops (sweet potato, cassava, rice, maize, wheat, etc.) mainly in field, in vitro and seed genebanks. Reed et al. (2004) concluded that the relative costs of field and in-vitro collections are quite similar in many cases, but as collections increase in size, in vitro becomes a more economical option.

Survey respondents indicated that cryopreservation offers a more secure option than field collections in terms of protecting genetic diversity. However, few in-depth studies of losses from field genebanks have been conducted and no studies have specifically analysed coffee germplasm losses in field genebanks. While cryopreservation is presumed to be an effective alternative for coffee germplasm, providing more security and little if any loss once accessions are cryopreserved, this hypothesis has not been thoroughly tested in a developing-country genebank.

The current study aims at addressing these issues and increasing the understanding of cryopreservation as an alternative or complement to field conservation. The study addressed two major questions:

1. What are the real threats to and losses of coffee germplasm as seen in the field genebanks where coffee is currently being conserved around the world?
2. How expensive is cryopreservation of coffee germplasm, and how do the costs compare with those of maintaining coffee field collections?

3. How effective is cryopreservation in terms of coffee accession viability and security?

3. METHODS

3.1 Choice of collections used in case study

The study compared the costs of cryopreservation and field genebanks, using as a case study the coffee collections held at CATIE (Centro Agronómico Tropical de Investigación y Enseñanza) in Costa Rica. During the period from 1999 to 2000, research staff of IRD (France) and CATIE (Turrialba, Costa Rica), with financial support from Bioversity developed and optimized the technology for cryopreservation of coffee seeds and established the first coffee cryobank in the world with a subset of CATIE's coffee core collection, comprising 63 genotypes (Dussert et al., 1997c, 1998, 1999a, 1999b, 2002; Vásquez et al, 2005). Staff members from various institutions in Latin America were trained in this technology. The cryopreservation protocol, developed jointly by Bioversity, IRD and CATIE (see below), was first applied to a subset of the CATIE coffee core collection conserved under field conditions. It provides the ideal situation whereby a cryopreservation protocol has been developed and applied to a species that is still being conserved under field conditions in the same location. Furthermore, because Costa Rica is a tropical developing country, this case can be used to assess the appropriateness of this methodology in the context in which most coffee germplasm originated and is conserved.

The study also compared the costs of cryopreservation of coffee germplasm in CATIE with those associated with cryo-collections of other species, which were obtained from several organizations holding cryopreserved collections, both in developed and developing countries (France, USA, Brazil). In addition cost data was also obtained on the coffee field collections in other major coffee collections in Madagascar, Ethiopia, Kenya and Brazil. These coffee collections are described below.

3.2 CATIE coffee field collection in Costa Rica

The Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) field collection of coffee was first established in 1949 in Turrialba, Costa Rica by CATIE's predecessor, Instituto Interamericano de Cooperación para la Agricultura (IICA) – the collection currently covers approximately 9 ha. Detailed information about the history and sources of introduced accessions in CATIE was documented by Anthony et al. (2007). The accessions introduced in 1949 represent 1.4% of the total number of conserved accessions. The largest introductions took place over 20 years between 1951 and 1970 (Figure 1), when the principal FAO coffee collecting missions occurred (Fernie, 1968); these constitute 58.3% of the living accessions in the collection. The acquisition of new

accessions decreased during the following decade, and increased again between 1981 and 1990, when CATIE received accessions from the ORSTOM collecting mission.

3.3 Madagascar field coffee collection

Madagascar's coffee collections are unique in that they hold a wide diversity of species, most of which are endemic to the island (see Table 1). Two collections were established in the early 1960s at Kianjavato and Ilaka Est, following collecting missions by the French institutions ORSTOM and CIRAD. Information is available on those accessions introduced from the time of establishment of the collections' until 1971 (thereafter, no records are available, as many documents were lost when the French left the island during the socialist revolution in 1972). Between 1960 and 1971, 319 accessions were inventoried (254 at Kianjavato and 65 at Ilaka). The Kianjavato collection consisted of 6,218 trees held over an area of 11 ha. The second collection in Ilaka Est is comprised mostly of accessions that had been conserved at Kianjavato; few accessions of Ilaka are unique.

3.4 Jimma Agricultural Research Centre, Ethiopia.

The major coffee germplasm field collection in Ethiopia is maintained at Jimma Agricultural Research Centre and its sub-centers Gera, Haru, Awada, Agaro and Mechara, which are located in the major coffee growing areas. Although early collections were established in the 1950s, the principal coffee collection was established following FAO and French Collecting missions in the 1960s (Figure 1). Since 1970, there has been a long-term national collecting programme in which 50 to 100 accessions per have been collected. In 1998 and 2002, more intensive collecting took place in efforts to save the area's coffee germplasm, which was endangered by drought, competition with chat (*Khata edulis*) and other threats. More accessions were added to the collection in the following decade as accessions were collected from the highlands of Ethiopia and added to the collection. Presently, there are over 5,000 *arabica* coffee accessions collected from different coffee growing areas and maintained at Jimma Agricultural Research Center and its sub-centers.

It is known that Ethiopia is the center of origin and center of diversity of *C. arabica* L. Since the diversity in the coffee forest ecosystem is so great, it is not possible to capture

all the available genetic variability through collection. For this reason, in addition to ex-situ conservation efforts, an in-situ conservation programme has been supported by the European Commission (EC). In this programme, three forest coffee conservation sites – Boginda-Yeba, Kontir-berhan and Geba-dogi – covering 21,000 ha have been identified and protected. The identification of more in-situ conservation sites continues, but these efforts are constrained by a limited budget.

3.5 Coffee Research Foundation, Kenya

Introduction of coffee germplasm into Kenya started in the early part of twentieth century, but these samples were subsequently discarded as they were poorly adapted to Kenyan growing conditions. The coffee germplasm collection was re-established in the early 1960s, with most introductions occurring between 1961 and 1980 (Figure 1). These introductions were obtained during a 1964 FAO collecting mission and two ORSTOM collecting missions in 1966 and 1977. The latest collecting mission sought to enrich the collection with indigenous coffee species from Kenya.

3.6 Brazilian coffee collections

There are several coffee field collections in Brazil. The principal *Coffea* genebank in Brazil, located at the Fazenda Santa Elisa at Campinas, São Paulo, is maintained by the Instituto Agronômico de Campinas (IAC). It was established in 1932 and is composed of 13,900 accessions, 65% of which are hybrid varieties, and including 3,800 accessions of *C. arabica*. It also contains 1,000 accessions of *C. canephora* and 14 other *Coffea* species as well as *Psilanthus* species. The collection is maintained in an area of 40 ha. A second collection, held by the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper) is located at the Fazenda Experimental de Marilândia, in Marilândia, Espirito Santo State. This collection is essentially composed of clones of *C. canephora*. The genebank was established in 1985, with new accessions added each year since. Today, it contains 375 accessions with ten individuals of each accession, in an area of 2.2 ha.

Another comprehensive coffee field genebank was established in 1974 at the Instituto Agronômico do Paraná (Iapar), in Londrina, Paraná State. This collection has almost 3,000 accessions of *C. arabica* (wild material, cultivars and hybrids), *C. canephora* and other species of *Coffea*. A new collection was established in 2003 at the Fazenda Experimental de Patrocínio of the Empresa de Pesquisa Agropecuária de Minas Gerais. It

consists of more than 1,000 accessions of *C. arabica*, including 200 accessions of Timor Hybrids (see Table1). In 2002, a cryo-research collection of *Coffea* germplasm was also established at Embrapa Genetic Resources and Biotechnology, in Brasilia.

3.7 Collection of data

Data was collected from countries conserving coffee in field genebanks using a simple questionnaire that helped to ensure comparability and quality of data. The questionnaires were organized to collect information about number of accessions conserved over time, the number of accessions lost over time (and causes of the loss) and number of individuals per accession. Data was also collected on costs of field and cryopreservation, genebank establishment and annual maintenance, including field preparation and plantation, health management, weeding, fertilizer application, irrigation, and personnel. The institutes that were investigated, their locations and the type of collections they hold are listed in Table 2. Follow up contacts were made with each respondent via email or telephone to clarify responses or request supplementary data. Supplementary data on the status of the world's major coffee collections were also obtained from published literature and individual contacts with the collection holders (see Table 1).

3.8 Field collection losses

In order to assess the threats to and losses of germplasm in field genebanks, an assessment was made of losses of accessions and individual plants lost from field collections. Whenever possible, the number of accessions lost over time and the number of individuals maintained for each accession were documented.

The number of individuals per accession was a particularly important parameter as it permitted the evaluation of genetic erosion at the accession level. It is good practice to maintain at least ten individuals per accession in order to ensure that the accession adequately represents the extent of the genetic variation in the population. An analysis of the number of trees in an accession can be a good indicator of the extent of genetic erosion in the field collection.

3.9 Cost Study: CATIE coffee field collection and cryo-collection

The cost study was done within a production economics framework, based on and modified from methodology developed by Pardey et al. (2001), in which inputs (labour, equipment, etc.) are used to produce certain outputs (in this case, living plants and stored seeds). The estimated costs of the field collection and cryo-collection were obtained from CATIE and IRD (for the improved cryopreservation protocol cost only) are broadly categorized as 'establishment costs' and 'post-establishment' or 'maintenance costs'. This allows: (i) a comparison of the first year and post-first year costs within the same collection; (ii) a comparison of the establishment costs between the field and cryo-collections; and (iii) a comparison of the maintenance costs between the field and cryo-collections. Costs of items and activities in the collections are not based on a particular year, but are recent approximations, and are therefore not subject to inflationary discrepancies.

Maintenance costs include the cost of items that are incurred annually (staff time, chemicals, etc.) and capital items (major equipment) purchased once; the latter costs were annualized using Equation 1, which takes into account a theoretical interest rate of 2% and the service lives of the items. This enables the estimation of long-term costs for all items in the collections (see below). A depreciation profile of capital (major equipment) assumed the capital had lasted until the end of its service life and became unusable after that.

The various cost items in the collections are categorized as follows:

(i) Capital costs (major equipment, land, building) For the cryopreservation collection, these costs include the cost of major durable equipment and buildings such as liquid nitrogen storage tanks, liquid nitrogen transfer vessels, laminar flow cabinets and the laboratory building, which not only houses the cryo-collection but has multiple functions. The cost ascribed to the cryo-collection represents only a fraction of the total cost of the building and is calculated according to the space occupied by the cryo-collection itself. For the field collection, the only capital cost was land. Both the land and the laboratory space were taken as a one-time fixed cost of establishment. For subsequent years, their cost was annualized based on an inflation rate of 2%over a period of 50 years.

(ii) Quasi-fixed costs (educated labour) These costs are represent human capital input such as educated/scientific labour (managers/curators). These professionals are normally hired on multi-year contracts and their labour input does not fluctuate with the

size of the collection. The allocation of labour for various activities in the collection can differ, however.

(iii) Variable costs Variable costs are sensitive to the scale of the collection's operation and include costs of propagation, regeneration and – in a field collection – low-skilled, unscientific or seasonal labour. In a cryo-collection facility, these include minor equipment such as lab materials and liquid nitrogen.

In-perpetuity costs are important as germplasm conservation is a long-term endeavor that requires a long-term perspective, in spite of the fact that genebanks are normally financed on a short-term basis. These costs include the amount of money required to fund the genebank activity in perpetuity and are based on the present values of the costs needed to fund the genebank at current levels in the long term at a theoretical discount rate (in this study, 4%).

Method of calculation

ANNUALIZED COST OF CAPITAL (DURABLE MACHINERY)

Y = [1- a / 1- an] x **Equation (1)**

(**Y**= *annualized cost;* **a**= *1/1+r;* **n**= *service life;* **r**= *interest rate;* **X**=*cost of item*)

Equation 1 is used to calculate the annualized user cost Y of an item that costs X dollars, purchased every n years. Annualized user cost values can then be used to calculate the present values of costs in perpetuity (see Equation 2 below) at theoretical interest rates.

COSTS IN PERPETUITY

This equation is used to calculate the present value of cost in perpetuity of a service costing X dollars purchased every year at time zero:

PV = [1 / 1 - a] Y **Equation (2)**

(PV= present value; **Y**= *annualized cost;* **a**= *1/1+r;* **r**= *interest rate*)

PRESENT VALUES OF COSTS IN THE FUTURE AT FIXED TIME PERIODS

$$PV = X + \frac{X}{(1+r)^{n}} + \frac{X}{(1+r)^{2n}} + \frac{X}{(1+r)^{3n}} + \ldots \quad \textbf{Equation (3)}$$

Using Equation 3, the present values of a cost (X) incurred in a specific time period were determined for each year at theoretical real rates of interest (2%, 4%, 6%) up to a specifically desired time period (30, 50, 70, 100, 150 years) and added up to provide the present value total for that time period.

4. CRYOPRESERVATION EFFECTIVENESS ASSESSMENT

The third area of analysis was to test the effectiveness of cryopreservation as a conservation technology for coffee. The major risks with cryopreservation were thought to be failure to appropriately apply the cryopreservation protocol during the establishment of the cryopreserved collections and during regeneration, and failure to constantly maintain the material at an adequate temperature during storage. To test whether the cryopreservation protocol was appropriately applied, and to assess losses in the event that it was not appropriately applied, a sample of the CATIE collection was regenerated to test the viability of the accessions.

In order to assess the viability of the cryopreserved coffee seeds, eight of the 63 accessions (12.7%) conserved in the cryobank were randomly chosen. For each of these accessions, ten seeds were thawed slowly in a water bath at 40 ºC for three minutes, followed by disinfection with commercial chlorine. Finally, the seeds were kept in sterile distilled water in a laminar flow cabinet for two days. After this conditioning of the seeds, the embryos were extracted and plated aseptically onto regeneration medium.

The re-warming of the accessions and extraction of embryos from the coffee seeds was conducted in groups of five during two distinct periods. The first experiment was initiated on 3 November with the following five accessions: 2711 A1, 17207 A3, 16723 A2, 4664 A4, and 21240 A1. The second experiment took place on 24 November 2006 comprising the following five accessions: 16723 A2 (replicate of first set), 16705 A2, 16692 A1, 21240 A1 PDRY (replicate of first set), and 4619 A1. Evaluation was done on three occasions at intervals of approximately ten days after culture.

5. RESULTS

5.1 Losses of accessions and diversity in field collections

Figure 1 gives the status of the field coffee collections in CATIE (Costa Rica), Kianjavato (Madagascar), Jimma Agricultural Research Centre (Ethiopia), the Coffee Research Institute (Kenya) and INCAPER (Brazil) in terms of the new accessions acquired since their establishment, and also shows the number of accessions lost during the same period.

The entire coffee collection at Illaka Est in Madagascar was lost in a severe tropical cyclone and was completely abandoned in the early 1990s due to lack of budget for its rehabilitation and maintenance. Madagascar's main collection at Kianjavato has also experienced heavy losses over the years. A complete inventory in 1982 revealed only 196 accessions, indicating a loss of 58 accessions between 1971 and 1982, among them two described species – *C. bengalensis* and *C. buxifolia*. Between 1982 and 1999, another 25 accessions were lost, including the Baracoffea accession. To date, the Kianjavato collection contains 3,668 individuals representing 173 accessions. Overall, Madagascar has lost 46% of its original accessions since its establishment, and 58% of its total number of trees.

In general, these losses can be attributed to the inadaptability of the species or accessions to the soil climatic conditions of Kianjavato. In addition, the genebank manager reported that between 1982 and 2000, the budgets allocated to the maintenance of the living collections were stagnant and inconsistent. In some years, no maintenance was undertaken at all, resulting in the loss of weak hybrid vigor accessions.

In Ethiopia, 657 accessions were lost over the period of study out of a total of 5,279 accessions planted. Reported causes of loss include poor early establishment, root and stem wilt diseases, or problems of adaptation of plants to the field-bank site because they were introduced from another locality to the research center.

At CATIE, the number of accessions lost over the different periods of ten years amounts to approximately 125 accessions out of 2,117, with most losses occurring between 1990 and the present (Figure 1). Losses were reported to be caused by: (i) age of the trees –

over 50% of the accessions were introduced before 1970 and are currently more than 35 years old (Table 9); (ii) waterlogging – the present site of the field collection is on flat land with structural soil problems, characterized by irregular patches of cemented soil layers at a depth of 30-80 cm; and (iii) management of the collection – a commercial style of management is used, not giving due attention to wild genotypes with different requirements than commercial varieties in terms of shade, pruning, fertilizer application, etc.

Kenya reported a loss of only 50 out of 2,557 total accessions, with no reported loss of accessions from the Kenya coffee collection since 1965. However, the data also shows that since 2000, there has not been any new introduction of coffee germplasm to the Kenya collection. The losses prior to 1965 occurred because accessions were removed from the field genebank for demonstration plots and to establish the tissue culture laboratory.

An analysis of the number of trees per accession can be a good indicator of the extent of erosion in a field collection. Information on the structure of the collections (the number of individuals per accession) was available only for CATIE and Madagascar, and is shown in Figure 2. Accessions maintained in Kianjavato are represented by a varied number of individuals: from one to as many as 156. The number of accessions with more than ten individuals has decreased from 118 to 82 between 1982 and 1999 (Table 4 and Figure 2), while all the categories with less than ten individuals have increased during this period. However, the 2006 inventory shows that accessions with more than ten individuals have increased while the other categories have decreased. This is the result of a donor-supported project that funded the replacement of missing individuals with at least ten individuals per accession, which has contributed about 90% of the maintenance cost of the collection since 2002.

At CATIE, a detailed analysis of genetic erosion in three areas of the coffee genebank between 1993 and 2002, stratified on the basis of the original source (cultivated or wild) and establishment date of the collection, demonstrated that genetic erosion averaged 3.6% for wild coffee accessions that had been established for 40 years in one section of the field genebank (Table 9). A higher rate of erosion (8%) was noted in another section of the field genebank composed of wild coffee accessions that had been established for 20 years in the field.

While the degree of erosion can be a function of level of management, it can also result from the genetic characteristics of the accessions and environmental events (such as storms and hurricanes). In the CATIE collection, the erosion rate, as shown by numbers of accessions represented by only one or two trees – the most threatened accessions – increased over the period 1993-2002 (Figure 2). The situation is different in Madagascar, however, where the number of accessions in the same category (1-2 and 3-4 individuals) increased from 1982 to 1999, but then decreased (Figure 2). An analysis of the data shows that 52% of the accessions lost were represented by less than three trees each. The fact that many trees (up to 156 individuals) were initially collected for each accession (see Figure 3) ensured their survival, except for a few that could not adapt to the Kianjavato's edapho-climatic conditions.

5.2 Cost of field and cryo collections

Tables 5 and 6 illustrate **total actual costs** of the CATIE coffee field collection and cryo-collection respectively. These include the first-year one-time fixed cost of establishment and the annual maintenance cost. These calculations are based on the current number of accessions in the field collection (1,992) and the current maximum capacity of the cryopreservation facility (300 accessions). For the field collection, the results show that the establishment cost for 1,992 accessions was US$138,681, with the cost of maintaining the collection amounting to US$30,343 annually. The costs of the cryopreserved collection were US$33,173 for the establishment cost and US$6,013.71 per year for the maintenance cost, for 300 accessions. The **per-accession costs** for the establishment of the field and cryo-collections are US$69.62 and US$110.58, and the per-accession costs for maintenance are US$16.00 and US$20.00 respectively.

It is not possible to directly compare the costs of establishment of the field collection with those of the cryo-collection because of the different numbers of accessions held in each. However, a projection of the cost of cryo-collection for a collection of 1,992 accessions shows that the establishment cost would be US$113,296 with a per-accession cost of US$56.88. The maintenance cost for 1,992 accessions in a cryo-collection would amount to US$29,601.58 with a per-accession cost of US$14.86.

Long-term cost projections for a cryo-collection and field collection with 1,992 accessions are presented as **in-perpetuity costs**, illustrated as total and per-accession costs at 2%, 4% and 6% (Table 7). At the highest theoretical interest rate (6%), the in-perpetuity cost of the field collection is US$557,339, while the cryo-collection cost stands at US$142,733. From a per-accession cost perspective, the field collection at a 2% rate of interest has an in-perpetuity cost of US$816 whereas the cryo-collection has an in-perpetuity cost of US$765 per accession. At a 6% rate of interest, the field collection has a per-accession in-perpetuity cost of US$283 while the cryo-collection per-accession in-perpetuity cost is US$265.

In addition, projection costs are also shown as **present values of costs incurred in future time periods** (up to 150 years), illustrated as total costs and per-accession costs,for the field collection and the cryo-collection with the same number of accessions (Table 8) at the three theoretical interest rates. At a 2% interest rate, the total cost of the field collection up to a period of 30 years is US$720,672 US, whereas the cryo-collection totals US$676,229. In 150 years at the same interest rate, costs are US$1,526,388 for the field collection and US$1,432,258 for the cryo-collection. At a 6% rate of interest, the cost in 30 years for the field collection is US$460,293, while the cost of the cryo-collection is US$431,908. In 150 years at the same interest rate, values are US$557,241 for the field collection and US$522,877 for the cryo-collection. The per-accession costs in 30 years at a 2% interest rate are US$365 for the field collection and US$219 for the cryo-collection. In 150 years at the same interest rate, the per-accession cost of the field collection is US$774 versus US$265 for the cryo-collection. The value at a 6% interest rate for 30 years is US$283 for the field collection and US$725 for the cryo-collection (see Table 8).

5.3 Cryopreservation viability assessment

Results of the first viability experiment (3 November 2006) are given in Table 3 [this is an initial test, and other regeneration protocols will be applied in the future to reduce problems with contamination]. Twelve days after the initiation of the experiment (on 15 November 2006), a high rate of infection with fungi and a mixture of bacteria with fungi was noted for ten vials, 20% of the total number of samples. Only one accession (2,711 A1) was absolutely free of infections through the first two evaluation dates. Green embryos, a sign of rapid regeneration, were noted in the second and third evaluations (27 November and 4 December 2006) for accessions 17207 A3 (10% of embryos) and 4664 A4 (40% of embryos).

The results of the second experiment are preliminary, as only one evaluation has been made at ten days after initiation. Fungal contamination was very high in this second set, affecting 11 vials or 22% of the cultures, while a mixture of bacterial and fungal contamination affected only three vials of one specific accession (16705 A2). As in experiment one, only one accession (4619 A1) was absolutely free of infection at the first evaluation date. The chances of recovery of the embryos in the second set of accessions are much lower than with the previous batch: 36% of embryos (versus 48% in the first experiment) had growth potential at the first evaluation date. Only one out of ten vials showed white, turgid embryos (accession 21240 A1 PDRY).

The viability experiments are still not yet conclusive and further observation is needed before and conclusions can be made.

6. DISCUSSION

The extent of genetic erosion in field coffee collections is highly variable from one genebank to another (see Figure 1) and is highly dependent on the resources available for maintaining the field collections, on the species being conserved and their origin. Madagascar has been the most heavily affected by genetic erosion, but still has the most diverse collection in terms of species (Table 1). The INCAPER collection in Brazil, which holds clones of *C. canephora,* has not lost a single accession since its establishment. This is because INCAPER has access to secure funding, which allows it to maintain the safe standard of at least ten individuals per accession. By contrast, Madagascar has suffered a lack of adequate financial resources to maintain its collections over time.

At CATIE, the differences in genetic erosion were attributed to the genetic characteristics of the accessions conserved (wild material). Most of the lost accessions disappeared in the early years after introduction (Bertrand et al., 1993). The greater rate of survival recorded in the oldest plot (Section A) can be explained by the higher initial number of trees per accession in this part of the genebank (Anthony et al., 2007) and the fact that this plot contained mainly cultivated accessions, which typically adapt more easily to new environments.

Reported losses of accessions were attributable to both human and natural causes. Madagascar reported stagnant, unstable budgets, which meant that maintenance could

not be conducted on schedule. CATIE reported that genebank management (in terms of fertilizer, water and other inputs) was not customized to the variability within the collection, and instead was uniform for all coffee varieties, which resulted in losses. Natural causes of losses included a tropical cyclone in Madagascar, root and stem wilt disease in Ethiopia, and aging of trees and poor drainage at CATIE. In both Ethiopia and Madagascar, losses resulting from inability of plants to adapt to the different site conditions in the field genebank were also reported.

The constraints to the effective management of field genebanks are generally well understood and have been discussed by various authors (Dulloo et al., 2001; Engelmann and Engels, 2002; Reed et al., 2004). One of the greatest limiting factors is a lack of financial resources to maintain genebank management activities, which in the case of field collections includes weeding, irrigation, fertiliser application, pest and disease control, shade management, maintenance of fencing to protect collections and other general maintenance. In Madagascar, the budget allocated for maintenance of the collection was negligible and no maintenance work was carried out for several years, leading to genetic erosion. Management and control measures can reduce genetic erosion caused by pests, diseases and other natural hazards such as drought, weather damage, human error and vandalism. In view of the frequent deaths of individual trees, there is a need to continuously regenerate accessions from other extant individuals; this adds to the cost of maintenance.

Compared with establishing field collections, cryopreservation theoretically offers a more secure means of conservation since there is no loss in germplasm viability during storage. Losses can occur during the recovery of the accession from cryopreservation or during the cryopreservation process, however. The trials conducted in this study showed a high prevalence of fungal and bacterial infections in the CATIE samples, especially the first set of five accessions, which severely affected the survival rate of the extracted embryos. In most cases, the infections seem to be directly linked to the extracted embryos. While a mixed bacterial/fungal infection eliminated 20% of the cultures (10 vials) in experiment one, only three vials of one accession were affected by this combination of pathogens in the second experiment. The high infection rate and the relatively low recovery rate of the extracted embryos in both sets of experiments is a reflection of the technique used. The risk of contamination during the in-vitro culture is high and can lead to possible losses of plantlets.

This low rate of recovery is not the result of a loss of seed viability during cryo-storage, but to the fact that many embryos were lost to contamination after they were introduced in-vitro. Experience gained at IRD has shown that slow cooling of seeds using a programmable freezer followed by re-warming, allows for direct germination of the seeds in the greenhouse, without the need for in-vitro culture. Freezing is more complex, but regeneration is very simple. Dussert et al. (2003) developed a probabilistic tool demonstrating that for any accession, a 95% probability of recovering at least ten plants (equivalent to ten trees per accession in the field genebank) with a mean seed survival percentage of 50% can be obtained from 45 seeds. This cryopreservation protocol developed for coffee seeds yielded a very high rate of recovery (Dussert et al., 2007) and demonstrates that, provided adequate replications of the accessions are made, all accessions can be safely regenerated. Experiments carried out in Brazil using a similar cryo-storage protocol for seeds of several *Coffea* species led to the establishment of 30 *C. arabica* accessions that had been stored for up to three years. Their viability, checked after two years of storage, demonstrated that the protocol is valid and that the seeds retained at least 80% of the initial viability. Seeds of other species such as *C. racemosa* and *C. eugenioides* have also behaved well in the cryogenic temperature.

This study showed that cryopreservation costs less (in perpetuity, per accession) than conservation in field genebanks. The initial cost for the establishment for a cryo-collection with 1,992 accessions (US$113,296 or US$56.88 per accession) is less than that of a field collection of the same size (US$138,681 or US$69.62 per accession), which is in the same range (US$50-75) as reported by USDA in their establishment of a cryopreserved temperate fruit collection in Corvallis (Humming and Reed, 1999). However, Trontin (pers. com.) reported that the establishment of an elm cryobank in France, which includes 200 bud explants per clone/accession, is 31 Euros per accession. This can be about three times more costly than direct establishment of three ramets/clones in the field (integrated, actualized cost over one century). Charrier et al. (1989) calculated the annual cost of establishing and maintaining the field collections of *Coffea* germplasm at Divo (Côte d'Ivoire) as being about US$80 per genotype, representing 25% of the total budget of the coffee genetic resources programme in Côte d'Ivoire over a period of 20 years.

A comparative analysis of the costs of cryopreservation and field genebanks showed that there are economies of scale associated with cryopreservation, since the more accessions there are in cryopreservation storage, the lower the per-accession cost. The current CATIE cryo-collection, with 63 accessions of coffee, is not cost effective – the cost per accession for maintaining this collection is greater than US$110.58 (calculated based on 300 accessions). However, if the size of the collection were increased to the size of the field collection (1,992 accessions) the establishment cost per accession would be reduced to US$56.88, while the annual maintenance cost per accession would be US$14.86. The most significant cost increase (excluding labour cost) would be the capital cost of purchasing additional liquid nitrogen storage tanks.

It must be noted that although costs of capital, small equipment and material in the cryo-collection are annualized, the actual purchasing of equipment spans can many years and even decades. By contrast, in the field collection, significantly higher maintenance costs are incurred perpetually on an annual basis. Cost dynamics, therefore, differ over time between the two types of collections. Field collections require higher investments more immediately and in *shorter periods* of time to sustain themselves. This makes them vulnerable to losses if financing for annual maintenance is unavailable for one or more years, as was the case in Madagascar. This can be especially serious when field collections hold rare or endangered germplasm, where losses caused by sporadic and insufficient financing can lead to extinction. The lower annual maintenance cost of cryo-collections, along with the longer intervals between required capital expenditures, make this conservation technique less vulnerable to the funding hurdles and inconsistencies that are a reality of the environment within which many genebanks operate today.

Total costs illustrate greater savings for cryo-collections compared to field collections. The costs of conservation depend on the type of operation – some accrue costs annually (storage) while others incur costs periodically. In addition, conservation costs critically depend on: (i) the biological characteristics of the type of crop being conserved; (ii) the conservation methodology used (for example, vegetatively propagated species maintained in vitro as clones or in field genebanks are much more expensive to conserve than stored seeds); (iii) institutional differences (such as the local wage structure, the composition of the labor force and cost-sharing opportunities with other local activities); (iii) local climate, which may be inappropriate for regeneration of some accessions; and (iv) the general state of infrastructure available to the genebank (electricity supplies,

communications, etc.) – see Koo et. al. (2003) for a detailed explanation. Annual and long-term estimates are sensitive to other factors such as crop composition, size of the holdings, the number of samples distributed annually from genebanks, the rate of interest used to calculate the present value of distant future costs and various conservation protocols (especially the frequency with which aging seed samples are tested for viability and regenerated) (Pardey et al., 2002). As already mentioned , the number of accessions in a collection also affects cost, as cost per accession is higher for lower numbers of accessions than for larger numbers due to economies of scale.

In addition to cost, cryopreservation has many other advantages over field collection. It can be regarded as a clean, environmentally friendly technology. Other than liquid nitrogen, a natural product, it does not use any pesticide and fertilizers (pests and diseases are not present during the conservation phase). The space required for cryopreservation is much smaller than for a field genebank. As seen in the current study, the conservation of 1,992 accessions of coffee plants in the field required 9 ha of land while the same number of accession could be cryo-preserved within 10 m^2. The number of genotypes which a field genebank can hold is also restricted by the human, financial and land resources, limiting the genetic diversity it can conserve. The opposite is true for cryopreservation: the higher the number of accessions conserved, the lower the unit cost.

The cryo method is also more secure against unfavourable weather, vandalism, pests and diseases; in a field collection, accessions are grown in a monoculture, making them susceptible to pests and diseases. This vulnerability is accentuated by the fact that many accessions may not be adapted to the local environments of field genebanks. Local climate and other environmental conditions represent strong selection pressures on individuals in field genebanks, and contribute to skewed genetic erosion in field collections. These problems can be mitigated to some extent by cryopreserving a core collection – a subset of accessions of a large germplasm collection chosen to represent the genetic variability of the whole germplasm collection (Frankel, 1984) – or by duplicating the collection in diverse eco-geographic sites.

7. CONCLUSIONS

In this study, coffee was investigated as a test case, but the results are relevant to other species that are difficult to conserve using the traditional conservation method of seed drying and low-temperature storage. The underlying objective of the study was to assess

the potential impact of the wide-spread adoption of cryopreservation methodology for more species and in more genebanks. Over the past fifteen years, Bioversity and many of its partners have invested in the development and adoption of cryopreservation methodologies by developing, testing and documenting protocols, training technicians and scientists, and supporting the acquisition of equipment for cryopreservation. Once adopted, cryopreservation is expected to result in improved conservation of difficult species in terms of: (i) greater efficiency of conservation (lower costs and more efficient use of a complementary range of different conservation methods); (ii) more effective and secure conservation (better viability over the long term, less overall loss of germplasm to pests, diseases and natural disasters, and maintenance of accession purity); and (iii) a wider range of diversity conserved (with a greater number of accessions conserved).

The results of the study indicate that in the long term, the costs of cryopreservation are lower than those of maintaining field collections. For base collections, cryopreservation offers an important complementary ex-situ option to field collections. In field collections, losses of accessions and the genetic diversity within them were observed, particularly in Madagascar, which has faced the most uncertainty and wavering financial and political support for its field collection over the years. Events in Madagascar also demonstrated that it only takes one catastrophic event such as a typhoon to destroy an entire collection maintained in the field.

Madagascar is believed to hold most of the untapped potential from coffee diversity. As more threats are seen to wild germplasm through land conversion, global warming and other forces, it may not be possible in the future to re-establish from the wild the diversity lost from field collections struck by disasters, diseases and insect attacks. This study shows that the costs of cryopreservation should not be considered to be prohibitive to establishing additional cryo-preserved collections of coffee and that environmental threats – by their nature random and difficult to predict – can cause serious losses to genetic diversity. If cryopreservation is adopted as a complement to field collections, either individual countries or those operating collaboratively would be required to add additional cryopreservation costs to their current costs of field conservation. Alternatively, they could reduce their field collections and replace some of them with cryo-preserved collections. Further studies are needed in order to establish the optimal balance between cryopreservation and field collection.

Given the importance of the genetic diversity of coffee to consumers worldwide and to coffee producers, many of whom are poor farmers dependent upon the coffee trade for their livelihoods, policy makers from coffee- producing and conserving countries should lay the groundwork for establishing additional cryo-collections in order to ensure the future health of the crop. As has been done with other crops (such as *Musa*), a regional or global cryo-preserved collection might be established for coffee germplasm, by which the costs of cryo-conservation and benefits flowing from the germplasm conserved could be shared among partner countries.

This study raised questions about the application of the cryopreservation protocol to coffee, since a significant percentage of the germplasm cryopreserved was lost as a result of infection in the thawing and regeneration processes. In order to ensure that an adequate protocol is available, additional research should be conducted and the results made available to the coffee conservation community.

Furthermore, policy makers and researchers should recognize that this study has broader implications, since many of the world's most important crops cannot be conserved *ex situ* using conventional methodologies. The costs of conservation in field genebanks is likely to be greater for short-lived crops like annual vegetables since they require more maintenance and more frequent regenerations in the field. Under these conditions, cryopreservation is likely to be even more cost efficient and should be considered strongly as a conservation option.

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Table 1. Status of the world's major coffee field genebank collections

Country	Institute	Total Nos. of accessions	Taxa (nos of accessions in brackets)	Source
Brazil	Instituto Agronômico de Campinas (IAC)	13901	*C. arabica* (4000), *C. canephora* (1000), Other *Coffea* species including *C. brevipes*, *C. congensis*, *C. eugenioides*,, *C. kapakata*, *C. liberica*, *C. racemosa*, *C. salvatrix*, *C. stenophylla* (14) and *Psilanthus bengalensis* (3), other material (84) and hybrids (9,000)	IAC, 2006
	Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural(INCAPER)	375	*C.canephora clones*	INCAPER, 2006
	Instituto Agronômico do Paraná (IAPAR)	2976	*C. arabica* (more than 1,000), *C. canephora* (50), other *Coffea* species including *C. dewevrei, C. eugenioides, C. kapakatta, C. stenophylla, C. racemosa, C. liberica* and *C. congensis* (8) and hybrids (1000).	IAPAR, 2006
	Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG)	1160	*C. arabica (*1160), including 200 accessions of Timor Hybrids	EPAMIG, 2006
Cameroon	Institut de la Recherche Agronomique	1552	Acessions composed mainly of *C. arabica*, *C. brevipes*, *C. canephora*, *C. congensis, C. liberica, Psilanthus* spp.	Bettencourt and Konopka, (1988), FAO (1996)

	(IRA)			
Colombia	Centro Nationale de Investigaciones de Café Pedro Uribe Mejia (CENICAFE)	1804	*C. arabica* (886), *C. canephora* (58), *C. congensis*(8),*C. eugenioides* (8), *C. kapakata* (2), *C. liberica* (19), *C. racemosa* (5), *C. salvatrix* (1) and *C. stenophylla* (1), *Psilanthus. travancorensis* (1), *.Psilanthus bengalensis* (1), Interspecific hybrids (102), and Intraspecific of *C. arabica* (712)	Bettencourt and Konopka, (1988)
Costa Rica	Centro Agronómico Tropical de Investigación y Enseñanza (CATIE)	1992	*C. arabica* (1809), *C. canephora* (88), *C. liberica* (25), *C. sessiliflora* (16), *C. brevipes* (14), *C. pseudozanguebariae* (10), *C. racemosa* (9), *C. eugenioides*, (8), *C. congensis* (5), *C. bengalensis* (3), *C. salvatrix* (2), *C. stenophylla* (1), *C. klainii* (1), *C. travancorensis* (1)	CATIE (2006)
Cote D'Ivoire	Centre National de la recherché Agronomique (CNRA)	8003	*C. arabica* (1787), *C. brevipes* (126), *C. canephora* (811), *C. congensis* (1598), *C. costatifructa* (46), *C. eugenioides* (1000), *C. fadenii* (4), *C. heterocalyx* (12), *C. humbotiana* (182), *C. humilis* (462), *C. kapakata* (2), *C. liberica* var *dewevrei* (738), *C. liberica* var liberica (253), *C. millotii* (1), *C. perrieri* (1), *C. pseudozanguebariae* (340), *C. racemosa* (66), *C. sakarahae* (11), *C. salvatrix* (44), *C. sessiliflora* (57) and *C. stenophylla* (216), *C.* sp (551), *Psilanthus* sp (sub-genus *Afrocoffea*) (23), *Psilanthus* sp. (sp (sub-genus *Psilanthus*) (52), *Psilanthus bengalensis* (3), *Psilanthus ebracteolatus* (22), *Psilanthus mannii* (196)	Anthony (1992)
Ethiopia	Jimma Agricultural Research Center	4652	All *C. arabica* except six of the are diploid species *[C.canephora (1), C.kapakata (1), C. eugenoiodes (1), C.racemosa (1), C.stenophyla (1), and C.congensis (1)]*	JARC (B. Bayetta, personal communication) (2006)

	(JARC)			
India	Central Coffee Research Institute, Kamataka	575 +	*C. abeokutae, C. arabica* (329), *C. canephora* (240), *C. congensis*, *C. eugenioides*, *C. liberica*, *C. racemosa (6)*, *C. salvatrix*, *C. stenophylla* and *C. zanguebariae*	Bettencourt and Konopka, (1988)
Indonesia	Indonesian Coffee and Cocoa Research Institute (ICCRI)	1637	*C. arabica (186), C. canephora (1296), Other coffee spesies including interspesific species (155).*	ICCRI (2006)
Kenya	Coffee Research Foundation	2507	*C. arabica* (592), *C. eugenioides* (28), C. *zanguebariae* (13), *Coffea* sp. (1)	CRI (C. Omundi, personal Communication) (2006)
Madagascar	Kianjavato, FOFIFA	171	*C.abbayesii* (1), *C.alaotrensi* (1), *C.alleizetti* (1), *C.ambodirianensis* (1), *C.andrambovatensis* (2), *C.ankaranensis* (4), *C.arenesiana* (1), *C.augagneuri* (3), *C.bertrandi* (3), *C.betamponensis* (1), *C.boiviniana* (7), *C.bonnieri* (1), *C.commersoniana* (1), *C.coursiana* (1), *C.costei* (1), *C.daphnoÎdes* (1), *C.dolichophylla* (3), *C.dubardi* (11), *C.ebracteolata* (1), *C.eugenioides* (1), *C.farafanganensis* (1), *C.heimii* (2), *C.homollei* (3), *C.humblotiana* (2), *C.jasminoides* (1), *C.jumellei* (1), *C.kianjavatensis* (2), *C.lancifolia* (3), *C.mangorensis* (3), *C.mauritiana* (8), *C.millotii* (2), *C.mogeneti* (2), *C. perrieri* (8), *C.pervilleana* (3), *C.ratsimamangae* (4), *C. resinosa* (12), *C.richardii* (4), *C.sahafariensis* (3), *C.sakarahae*(4), *C.spathilifolia* (1), *C.tetragona* (1), *C. tsaratananensis* (2), *C.tsirananae* (2), *C.vatovavyensis* (3), *C.vaughanii* (2), *C.vianneyi* (6), *C.vohemarensis* (1), *C.sp.* (35), and *Paracoffea* (4)	FOFIFA (J.J. Rakotomalala, personal communication) (2006)

Tanzania	Tanzania Agricultural Research Organiation	110	*C. arabica* (42), *C. canephora* (19), *C. congensis* (5),*C. eugenioides* (10), *C. kapakata* (1), *C. liberica* (24), *C. ligustroides* (1), *C. mufindiensis* (1), *C. racemosa* (2), *C. salvatrix* (1) and *C. stenophylla* (1), *C. zanguebariae* (1) and *Nostolachma khasiana* (1)	Bettencourt and Konopka, (1988). Note: *C. congo*, *C swynnertonii* and *C. khasiana* have been renamed *C. congensis* and *C. racemosa* and *Nostolachma khasiana* respectively (D. Bridson, personal communication)
USA	United States Department of Agriculture	300	*C. arabica* (292), *C. canephora* (2), *C. racemosa* (5), *Psilanthus bengalensis* (1)	Bettencourt and Konopka, (1988)

Table 2. Institutions participating in study

Institution	Type of collection and species	Location
Tropical Agricultural Research and Higher Education Centre (CATIE)	Field collection coffee *C. arabica* and *C. canephora*, *C. liberica* wild African diploid coffee	Costa Rica
	Cryopreserved core collection of *C. arabica* coffee	Costa Rica
CENRADERU	Field collection coffee- wild Mascarocoffea species	Madagascar
Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper)	Field collection coffee *C. canephora*	Espírito Santo, Brasil
Embrapa Genetic Resources and Biotechnology (former Cenargen)	Cryopreserved collection coffee *C. arabica*	Brasília, DF, Brasil
Instituto Agronômico de Campinas (IAC)	Field collection coffee *C. arabica*	Campinas, São Paulo, Brasil
Coffee Research Foundation	Field collection coffee *C. arabica* and wild African diploid coffee	Kenya
Jimma Agricultural Research	Field collection coffee *C. arabica*	Ethiopia

Table 3. Viability assessment of first set of five accessions

INITIAL TEST, PROTOCOL 1. TESTS USING OTHER PROTOCOLS STILL IN PROGRESS.

Experiment 1:

Initiation of experiment: 3/11/2006
Date of first evaluation: 15/11/2006

Accession #	embryos apparently alive				% alive	dead embryos			% dead
	White	green	with fungi	with bacteria		without infection	fungal infection	Bacterial/ fungal infection	
2711 A1	4	0	2	0	60	4	0	0	40
17207 A3	0	1	1	0	20	0	4	4	80
16723 A2	5	0	0	0	50	0	5	0	50
4664 A4	6	2	1	0	90	0	1	0	10
21240 A1	1	0	0	1	20	2	0	6	80
					48				**52**

Date of second evaluation: 27/11/2006

Accession #	embryos apparently alive				% alive	dead embryos			% dead
	White	green	With fungi	with bacteria		without infection	fungal infection	Bacterial/ fungal infection	
2711 A1	4	0	2	0	60	4	0	0	40
17207 A3	0	1	0	0	10	0	5	4	90
16723 A2	5	0	0	0	50	0	5	0	50
4664 A4	4	4	0	0	80	0	2	0	20
21240 A1	1	0	0	0	10	2	1	6	90
					42				**58**

Date of third evaluation: 04/12/2006

Accession #	embryos apparently alive				% alive	dead embryos			% dead
	White	green	with fungi	with bacteria		without infection	fungal infection	Bacterial/ fungal infection	
2711 A1	4	0	0	0	40	4	2	0	60
17207 A3	0	1	0	0	10	0	5	4	90
16723 A2	4	0	0	0	40	1	5	0	60
4664 A4	3	4	0	0	70	1	2	0	30
21240 A1	1	0	0	0	10	2	1	6	90
					34				**66**

Experiment 2.

Initiation of experiment: 24/11/2006
Date of first evaluation: 04/12/2006

Accession #	embryos apparently alive				% alive	dead embryos			% dead
	white	green	with fungi	with bacteria		without infection	fungal infection	bacterial/ fungal infection	
16723 A2	3				30	5	2		70
16705 A2*	3				30	2	1	3	60
16692 A1	4		2		60	1	3		40
21240 A1 PDRY	1				10	4	5		90
4619 A1	5				50	5			50
					36				**62**

* one vial is missing

Table 4. Table showing the size categories of coffee accessions at Kianjavato, Madagascar

		Inventory years		
		1982	**1999**	**2006**
Accession Size categories	>10	118	82	105
	5-10	25	36	36
	3-4	13	18	13
	1-2	16	22	11

Table 5. CATIE Coffee Field Collection Costs (USD)

COST PERIOD / ITEMS	COST CATEGORY	COST (USD), 9 Ha
FIRST-YEAR (Establishment)		
Land	C	36,000
Labour for land clearing	V	4,797
Fencing		45,000
Labour for fence establishment	V	702
Land preparation	V	2,997
Shade establishment		
Initial propagation:	V	999
Nursery materials	V	11,250
Shade trees	V	90
Labour cost	V	4,995
Plastic bags	V	135
Fertilizer	V	225
Fungicides	V	225
Initial field planting:		
Labour cost	V	2,502
Fertilizers	V	855
Herbicides	V	252
Insecticides	V	657
Curator	QF	12,000
Technician	V	15,000
Labourer	V	Costs included in operations above
Total		***138,681***

cost per accession		***69.62***
Maintenance cost per year		
Curator	QF	12,000
Technician	V	15,000
Labourer	V	Costs included in operations
Plant health control	V	below
Weeding	V	
Fertilization	V	540
Repair fencing	V	648
		1,161
Re-establishment:		486
Propagation +Field planting	V	
Annualize land cost	V	
		508
Total		
		30343
Cost per accession (Total: 1,992)		***15.23***

Table 6. CATIE coffee cryo-collection costs (USD) for 300 accessions

COST PERIOD / COST	COST CATEGORY	COST (USD)
FIRST YEAR		
Lab space @450US$/m2- 16 m2	C	7200
Durable equipment		
3 liquid nitrogen transfer vessel ($1581)	C	4743
3 liquid nitrogen storage tanks ($2160)	C	6480
1 laminar flow cabinet	C	8100
Sub total		***19323***
Small equipment/materials**		
4 pairs of cryo-gloves ($ 204.50)	V	818
600 aluminum cryocanes ($1.40)	V	840
1800 cryogenic vials (5 mL) ($0.49)	V	980
3 marker sets of varying colors ($21.75)	V	65.25
1 Dewar flasks (2.5 L)	V	250
Sub total		***2953.25***

1 technician (preparation)3man-month	QF	1,500
1 technician (monitoring)3man-months	QF	2,000
Electricity	V	300
Liquid nitrogen replacement	V	3600
Vehicle to acquire liquid nitrogen	V	240
Sub total		***7,640***
<u>To add for seed drying prior to cryo</u>		
140 vials + screw cap ($1.45)	V	203
ammonium nitrate 15 kg ($165.40/2.5 kg)	V	992.40
plasticware	V	200
glassware	V	300
Sub total		***1695.40***
Control of seed viability*****		
Culture medium		200
100 autoclavable growth vessels ($3.48)		348
Sub total		***548***
Total		*39360*
Cost per accession		*131.20*

Maintenance cost per year		
1 technician (monitoring) 1 man month		500
Electricity		300
Liquid nitrogen replacement		3600
Vehicle cost to acquire liquid nitrogen		240
Durable Equipment (annualized costs*):		
3 LN transfer vessel (SL 15 yrs)		38.01
3 LN storage tanks (SL 15 yrs)		455.98
1 laminar flow cabinet (SL 15 yrs)		545.72
Total		**5679.71**
Cost per accession		***18.93***

Table 7. Field and cryo-collections, total and per accession maintenance cost, in perpetuity (per accession* costs are in bold) calculated on the basis of 1992 accessions for both field and cryo-collections

	2%	4%	6%
Field Collection	1608897 **816**	820222 **416**	557330.3333 **283**
Cryo-collection	240382.2 **765**	166023.2 **390**	142733.6 **265**

Table 8. CATIE Field Collection and cryo-collection. Present values (total and per accession costs, USD) in fixed time periods over time at theoretical interest rates. (Per accession costs are in bold) calculated on the basis of 1992 accessions for both field and cryo-collections

COST CATEGORY	RATE OF INTEREST	YEARS				
		30	50	70	100	150
Field Collection	2%	720671.8 **365.51**	1011146.9 **512.83**	1206628.32 **611.97**	1386816.17 **703.36**	1526387.78 **774.15**
	4%	567332.25 **287.73**	767547.835 **357.46**	767547.835 **389.28**	803981.571 **407.76**	817936.767 **414.84**
	6%	460293.48 **233.45**	527073.78 **267.32**	547896.198 **277.88**	555687.755 **281.83**	557241.16 **282.62**
Cryo-collection	2%	676229.4232 **218.86**	948791.5104 **250.61**	1132217.982 **260.51**	1301294.011 **264.21**	1432258.518 **264.95**
	4%	532346.00 **269.75**	661342.42 **335.12**	720214.70 **364.95**	754401.64 **382.27**	767496.25 **388.91**
	6%	431908.1037 **342.66**	494570.2031 **480.78**	514108.5419 **573.72**	521419.6093 **659.40**	522877.2195 **725.76**

Table 9. Genetic erosion estimated by the percentage of dead trees and lost accessions in three areas of the CATIE genebank, between 1993 and 2002. (from Anthony et al. 2007)

Section	**Age**	**Genetic origin**	**Dead trees**	**Lost accessions**
A	> 45 years	Cultivated	14.9%	2.0%
C	40 years	Wild	11.6%	3.6%
F	20 years	Wild	15.7%	8.2%

Table 10. Stakeholder responses

Potential impact domains	**Not significant**	**Somewhat significant**	**Very significant**	**Sample size**
Cryopreservation has the potential to reduce losses of genetic diversity due to diseases, insect attack, storms, natural disasters etc that occur in field genebanks or the wild	0% (0)	12% (3)	88% (23)	26
Amongst active cryo practitioners	**0**	**0**	**100% (13)**	**13**
Cryopreservation has the potential to ensure greater genetic integrity, since accessions are not contaminated by germplasm from neighboring plants	8% (2)	38% (10)	54% (14)	26
Among active cryo practitioners	**8% (1)**	**23% (3)**	**69% (9)**	**13**
Overall costs of conservation are potentially reduced through the use of cryopreservation	8% (2)	42% (11)	50% (13)	26
Among active cryo practitioners	**8% (1)**	**31% (4)**	**62% (8)**	**13**
Cryopreservation potentially facilitates the use of germplasm because accessions are more easily replicated and transferred as in vitro materials	19% (5)	31% (8)	50% (13)	26
Among active cryo practitioners	**8% (1)**	**23% (3)**	**69% (9)**	**13**

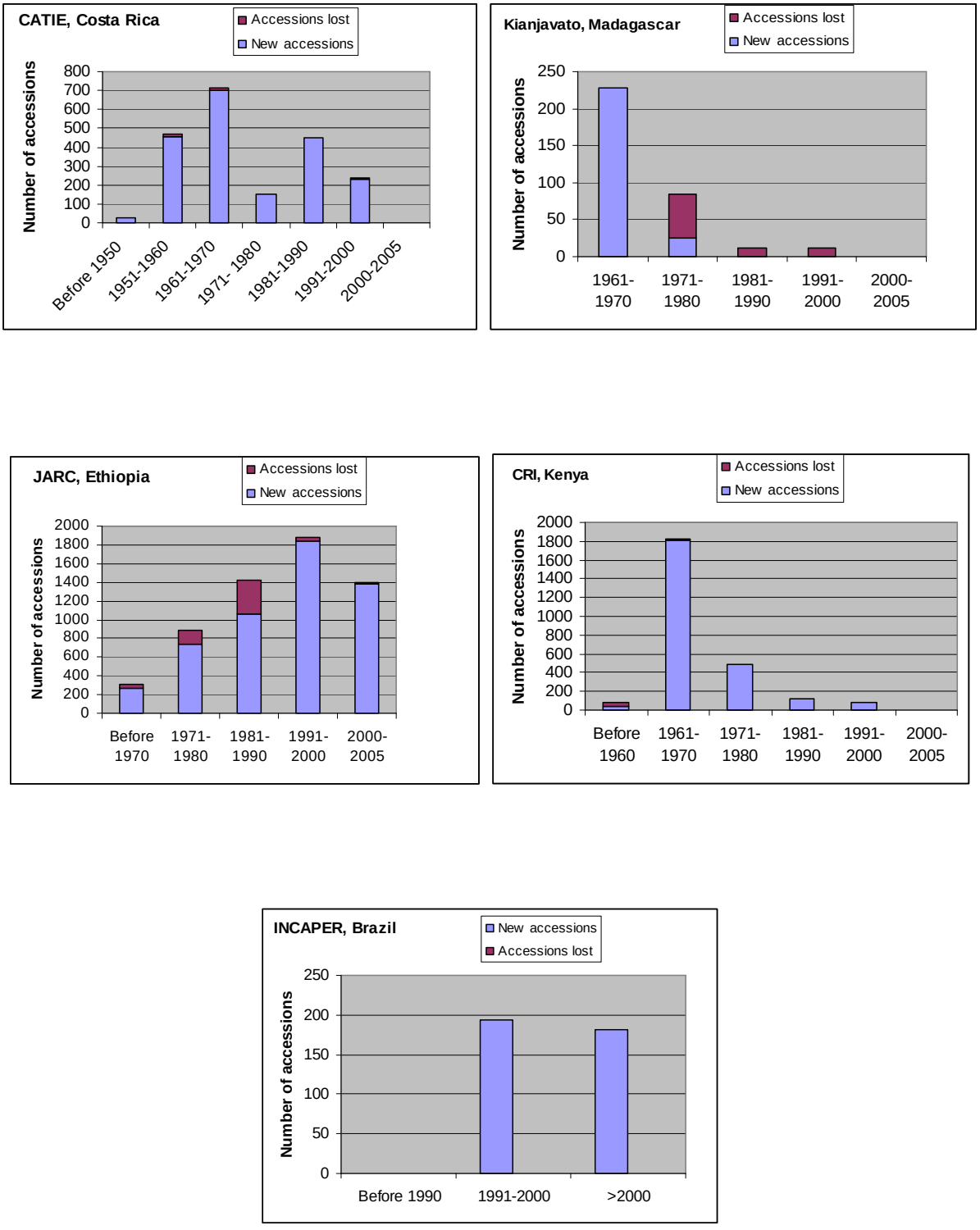
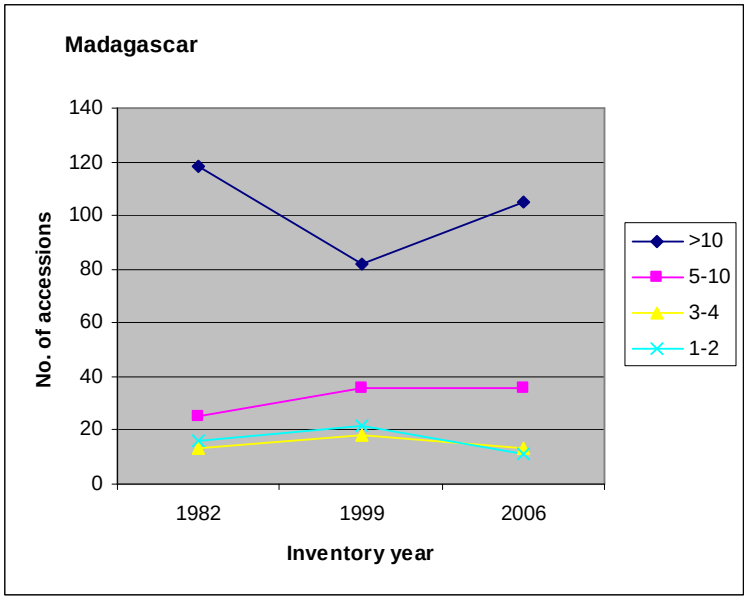


Figure 1. Status of field coffee collection in CATIE (Costa Rica), Kianjavato (Madagascar), Jimma Agricultural Research centre (Ethiopia), Coffee Research Institute (Kenya) and INCAPER (Brazil), showing the number of accessions acquired and lost over 10 years time periods, since their establishment.



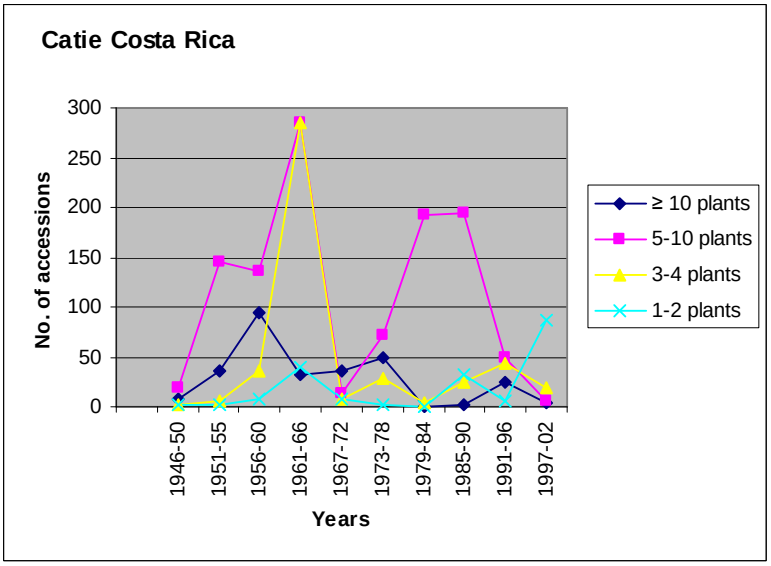


Figure 2. Structure of field coffee collection in Madagascar and CATIE, Cost Rica.

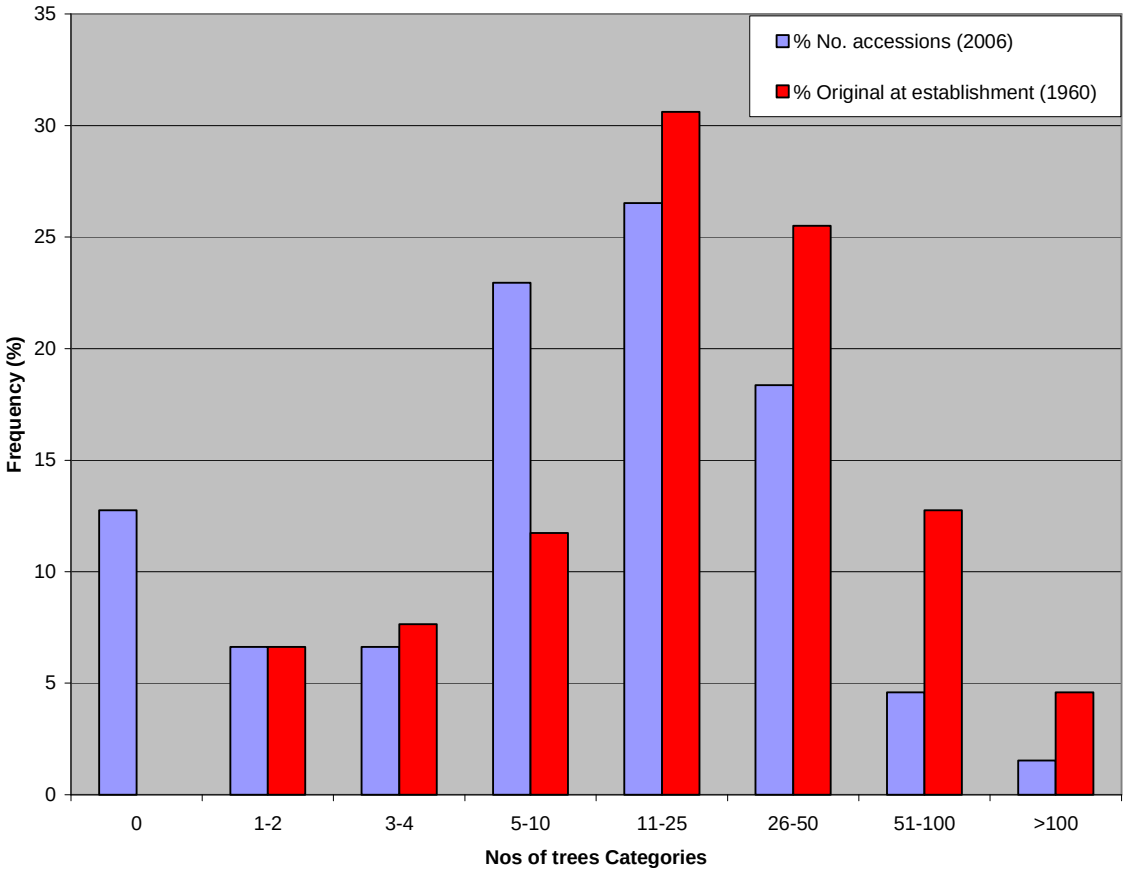


Figure 3. Percentage frequency of number of accessions in different size categories of the Kianjavato collection (Madagascar) in 2006 (blue bars) compared to the original number at establishment (1960)(red bars).