# VII. Conclusions and prospects

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Coffee has proven to be an interesting crop for developing complementary strategies and methods for *ex situ* conservation of genetic resources, as an example of a non-orthodox-seed species. As for many crops, coffee field genebanks are today facing many technical, financial and political challenges, which are difficult to resolve (Dulloo et al. 2001). For our review, an integrated action has been applied to the coffee genetic resources conserved in the CATIE field genebank, involving revision of passport data, diversity analysis, core collection construction, and transfer of a cryopreservation protocol from IRD to CATIE. This has contributed to establish, then to cryopreserve, a core collection that can be considered as being representative of *C. arabica* genetic diversity.

The detailed description of the CATIE coffee germplasm collection and its management has highlighted the main problems encountered frequently in large genebank operations. They include the absence of computerization, which can hide the potential presence of off-types, and the difficulties in locating a particular genotype in the collection. Another aspect of crucial importance is the loss of accessions, which might be considered of minor importance due to the relatively low average loss observed. It is in fact extremely serious as some groups, notably the accessions derived from wild coffee, face very drastic losses. This shows that germplasm collections should not be managed uniformly, but that they should be stratified according to the agronomic behaviour of conserved resources in order to adapt the cultural practices and management procedures.

Prior to evaluation, the accessions were classified according to their taxonomy and their geographical or genetic origin. This allowed structuring of the genetic resources and constructing a core collection for genotypic evaluation. The neutral marker analysis led to identification of genetic groups at intraspecific level, which groups were then used for constructing a representative core collection of the diversity conserved in the field genebank. Such an approach has provided genebank managers, breeders and research scientists with a manageable number of accessions for their work. A representative core collection of the Ethiopian accessions was thus constructed and the first world cryobank of *C. arabica* seeds was established at CATIE (Vasquez et al. 2005).

The cryopreservation protocol established in IRD Montpellier has been transferred without any major difficulty to CATIE and applied to a subset of the core collection defined, with plantlet recovery up to 74% of cryopreserved seeds (Vasquez et al. 2005). To our knowledge, this project represents the first example of a cryopreservation protocol being transferred and employed on a large scale in the laboratory of a developing country, in a plant genetic resources conservation context. This active cooperation between developed and developing world institutions has been

a key factor for the success of the project, some experiments being more easily carried out at IRD (e.g. development of cryopreservation protocols) and others at CATIE (e.g. their application to a large number of plants).

One of the significant advantages of this protocol over more classical ones is that no *in vitro* step is necessary at any stage of the protocol for most accessions, i.e. those which show seed survival after freezing, since seeds can be germinated under non-sterile conditions. In cases where survival of whole seeds is nil or very low, then excision and *in vitro* cultivation of zygotic embryos has to be performed, and produces excellent results, as all embryos remain alive inside the seeds, even if they cannot germinate (Dussert et al. 1997). Indeed, cryopreservation damages the endosperm but not the embryo, which conserves its germination and development capacities. The other current drawback of the method is that precooling of seeds to –50°C before their immersion in liquid nitrogen requires the use of a sophisticated programmable freezer. It is hoped that this step can be replaced by a more simple protocol (e.g. using a laboratory deep freezer), which would broaden its applicability.

The availability of the seed cryopreservation protocol as a new complementary technique should have consequences for the management of the coffee genebank. It should be tested on seeds of a broader range of coffee genotypes and species involving notably rare material, material little requested, material with specific characteristics, and material often requested. This should thus have consequences for the number of replicates of a given accession conserved in the field, if it is also stored under cryopreservation, depending on the decisions taken by the curator of the collection. Evaluation data indicated that some accessions present low polymorphism and others probably result from human duplications. Such accessions should no longer be maintained in the field genebank, but only in the form of cryopreserved seeds.

Various additional points should also be considered, such as the necessary safety duplication of the cryopreserved collection at at least one site other than CATIE, and a calculation of the number of seeds that should be stored per accession to ensure their regeneration. A specific field for cryopreserved material should be added to the general collection database. Procedures remain to be established for handling the material (retrieval upon demand for cryopreserved material, replacement of material taken from the cryobank, etc.). All these points could form the subject of a Technical Bulletin for laboratory daily use.

In conclusion, the conservation activities developed for coffee have demonstrated that it is possible to efficiently use cryopreservation for the long-term conservation of germplasm of a species with non-orthodox seeds, in the genebank context of a developing country. There is a huge number of species with non-orthodox seeds for which similar projects would be necessary in order to ensure the safe, long-term and cost-effective conservation of their genetic resources. Such a project is currently being implemented for *Citrus* spp., through collaboration between the Universiti Putra Malaysia (UPM), IRD and Bioversity (Hor et al. 2005). It is our hope that this publication will stimulate research in this area for additional non-orthodox-seed species and pave the way for application of such technologies to other species that have seeds that are difficult to conserve *ex situ*, or species that are at the moment solely dependent on conservation in field genebanks.

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