Cryopreservation at CATIE: an additional tool for the conservation of tropical agricultural crops and forest species

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Introduction

CATIE is a regional centre of research and post-graduate education in agriculture and natural resources. One of CATIE's missions is to improve and conserve genetic resources of Central America and the Caribbean region. Cryopreservation is a useful tool to support these objectives. This paper gives a brief overview of cryopreservation research activities currently ongoing in CATIE.

The collection of *Coffea arabica* at CATIE is one of the largest wordwide, with a total of 1820 accessions. To ensure long-term conservation of this collection, CATIE has initiated a collaborative research programme with ORSTOM, France and IPGRI on cryopreservation of coffee seeds. Since *Coffea arabica* is self-fertile, many cultivated accessions can be considered homozygous. For these accessions, seeds could be cryopreserved after self-pollination. A core collection to be cryopreserved will be defined using passport genetic diversity structure and characterization data.

CATIE is deploying important efforts toward the rescue, characterization and conservation of endangered tropical forest species. Many of these species have recalcitrant seeds. A project aiming at developing conservation methods for forest tree species has been initiated at CATIE. Different cryopreservation methods including encapsulation-dehydration, vitrification and slow freezing are tested with shoot-tips and embryonic axes.

Bananas and plantains are seriously threatened by pests and numerous viral and fungal diseases. Non-conventional improvement methods are being used for parthenocarpic edible bananas and plantains. A cryopreservation technique has been developed to conserve embryogenic cell suspensions of different *Musa* cultivars which are produced in CATIE with these programmes.

The objectives of CATIE's cryopreservation programme are:

- To rationalize CATIE's coffee collection by adopting ORSTOM's cryopreservation protocol for long-term storage of coffee seeds
- To develop cryopreservation protocols for shoot-tips, seeds and embryonic axes that could be applied to recalcitrant seeds and vegetative material from forest tree species
- To optimize a cryopreservation technique for embryogenic cell suspensions of *Musa* cultivars.

Cryopreservation of coffee germplasm

The first step of the seed cryopreservation coffee project is to transfer the cryopreservation protocol (Dussert *et al.* 1998) to CATIE. Experiments will be first performed with 2–3 genotypes and will focus on the optimization of the seed osmoconditioning procedure after thawing. Depending on the results, trials will be carried out under nursery/greenhouse conditions, either to achieve direct germination of osmoconditioned seeds or to improve the transfer of germinated embryos to nursery/greenhouse. Optimized protocols will then be applied to 13 *C. arabica* accessions chosen from wild and cultivated accessions, based on their variability in biochemical composition and size of their seeds. The first results will provide preliminary information on the feasibility of cryopreserving a broad genetic diversity using a single protocol.

Cryopreservation of tropical tree germplasm

In tropical forest tree species, several methods will be tested including encapsulation-dehydration, vitrification and slow freezing. Experiments will be carried out with 2–3 species to establish technical procedures. The first experiments are being performed for encapsulation-dehydration with mahogany (*Swietenia macrophylla*) shoot-tips. Trials are under way to determine the optimal conditions for pregrowth (sucrose concentration (0.3–0.75M) and pretreatment duration); bead desiccation under the laminar airflow (0, 2, 4, 6 h); direct immersion in liquid nitrogen; rapid thawing and recovery. The culture medium utilized for these experiments was developed for microcutting culture (Orellana 1997).

Limited survival (7%) of cryopreserved mahogany shoot-tips was achieved after pregrowth for 3 d in liquid medium with 0.5M sucrose, desiccation of beads for 6 h (27% water content), followed by direct immersion in liquid nitrogen and rapid thawing. Studies on *in vitro* conservation have not been reported for this species. However, Maruyama *et al.* (1997) reported shoot-tip encapsulation and storage at 12–25°C in *Cedrela odorata*. The best storage temperature was 12°C with a plant conversion rate of 80% after 12 months. Our objective for the short term is to improve the results obtained by performing additional experiments including modifications of the pregrowth and desiccation periods. Regrowth of mahogany shoot-tips after cryopreservation opens new possibilities that could be applied to other species.

Cryopreservation of *Musa* germplasm

Embryogenic cell suspensions of *Musa* cv. Dominico (AAB) were obtained according to Grapin *et al.* (1996) and Côte *et al.* (1996). Cryopreservation was performed using a method derived from Panis *et al.* (1990). For cryoprotective treatment, DMSO was added to the final concentration of 7.5% (v/v). For slow freezing the samples were cooled at 1°C/min to –40°C prior to transfer to liquid nitrogen (–196°C). Crystallization of the cryoprotective medium at –10°C was performed automatically by the apparatus (CRYOMED).

With *Musa* cv. Dominico, crystallization induction during freezing was indispensable to obtain regrowth after thawing (Fig. 1). Pregrowth of the cell

suspensions for 7 d with sucrose (0.39M) was the most efficient. Optimal sucrose concentrations during pretreatment were 0.39 and 0.53M. Cell regrowth was more efficient after high-density plating on solid medium. The optimized protocol was applied to various embryogenic cell suspensions of *Musa* and four out of five cultivars withstood cryopreservation (Dominico 1, SF 265, Curraré 3, Col 49 2.8). The efficiency of the protocol was calculated by the ratio of the number of embryos produced after plating a cryopreserved cell suspension against the number of embryos produced by a non-cryopreserved cell suspension (Fig. 2). The data ranged from 0 to 26% depending on the cultivar. This efficient system of quantification should be optimized for *Musa* and could also be applied to other species.

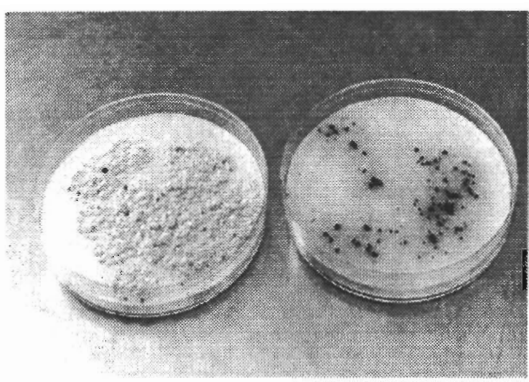

Fig. 1. Effect of crystallization induction in *Musa* cv. Dominico. Left: with induction, right: without induction.

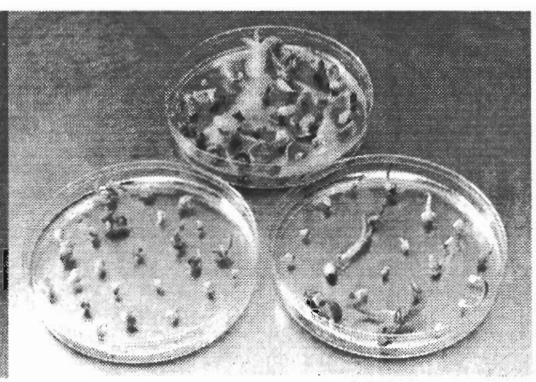

Fig 2. Germination of somatic embryos derived from cryopreserved cell suspension in *Musa* cv. Dominico. Top: Control, Right: with induction, Left: without induction.

Conclusion

The level of cryopreservation research in CATIE as well as the range of species covered by the programmes is expanding progressively. Mastering cryopreservation techniques will allow CATIE to conserve genetic resources of problem crops in a safer and more cost-effective manner.

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