Coffee Seed Cryopreservation: Current Research Progress

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SUMMARY

Viability of seeds stored in genebanks must be maintained for several years or even centuries. Because of the difficulties in storing the seeds, coffee germplasm is maintained in field collections, presenting significant problems, such as land and labor costs and susceptibility to environmental hazards and pathogens. Storage of *Coffea* species in *ex situ* genebanks may help to preserve the threatened diversity of this important genus. Since 1976 the International Plant Genetic Resources Institute has considered coffee as a high priority for genetic conservation. For non-orthodox seed species, cryopreservation is the only technique available for long-term germplasm conservation. The genebank of Embrapa Genetic Resources and Biotechnology, in Brazil has now established a program to cryopreserve genetic resources of *Coffea*. The protocol was first determined for *C. arabica* and *C. racemosa*. Seeds were first dried to 0.20 g/g (in equilibrium with 78-80% RH). Sufficiently rapid cooling and warming was achieved in hermetically-sealed foil-laminate bags containing 10-11 g (or 50 seeds) by plunging bags directly into liquid nitrogen – LN containers and placing bags removed from LN directly into a 40 °C bath. Successfully cryopreserved *C. arabica* and *C. racemosa* seeds showed minimal viability loss after two-year storage in liquid nitrogen. The same protocol is being adapted to other species of *Coffea.*

INTRODUCTION

Coffee is extensively cultivated as a cash crop in many countries, including Brazil, which is the main producer of coffee beans in the world. There is a great variability between and within species, which is of importance for breeding. A living collection of *Coffea*, comprehending most species of the genus, is maintained at the Instituto Agronômico de Campinas, IAC, Brazil. Significant problems appeared with the maintenance in field genebanks: i) genetic erosion in some species due to their poor adaptation to the local environment and to attacks by pests and pathogens; and, ii) significant labor costs and large space requirements. Thus, research for conservation of coffee genetic resources, in genebanks, as seeds, became a priority.

Seeds of *Coffea* species do not behave as the majority of seeds with respect to storage, according to the definitions of Roberts (1973). They are not recalcitrant, since they survive desiccation to water contents less than 0.20 g/g, and some seeds survive exposure to subzero temperatures, and they are not orthodox either, as the seeds do not survive complete desiccation and the combined effects of desiccation and low temperatures. Ellis et al. (1990; 1991) introduced the "intermediate" category of desiccation tolerance to describe seeds such as coffee, which can tolerate some drying but do not survive complete desiccation or the combined effects of desiccation and low temperature.

For non-orthodox seed species, cryopreservation is the only technique available for long-term germplasm conservation. In the case of intermediate seed-propagated species, seeds are partially desiccation tolerant and, therefore, the option which has to be always tested first is whole seed cryopreservation.

A certain amount of work has been carried out on the cryostorage of coffee seeds. Most of the papers reported coffee seeds as desiccation tolerant and liquid nitrogen sensitive. Nevertheless, Normah and Vengadasalam (1992) showed that seeds of *C. liberica* can survive storage in liquid nitrogen if the water content is around 17% (fwb). Eira et al. (1999) reported survival of *C. arabica* and *C. racemosa* seeds with 0.20g H2O/g dry mass after liquid nitrogen exposure. Dussert et al. (2001) reported that seed survival was strictly depended on avoidance of intracellular ice formation, and that 0.20 g/g corresponded to the seed unfrozen water content .According to those results, coffee seeds are not liquid nitrogen sensitive at appropriate water content, around 17% (fwb) or 20% (dwb).

OBJECTIVE

Our objective was to establish a program to cryopreserve *Coffea* seeds as a complementary strategy of long term germplasm conservation of that important genus.

The protocol was first determined for *C. arabica* and is now being adapted to other species, such as *C. racemosa*.

RESULTS

Successfully cryopreserved *C. arabica* and *C. racemosa* seeds showed minimal viability loss after two-years storage in liquid nitrogen.

The best protocol were obtained with seeds are extracted from ripe fruits at 0.20 g/g dw (in equilibrium with 78-80% RH), as reported by Eira et al. (1999) and Dussert et al. (2001). Sufficiently rapid cooling was achieved in by plunging the bags directly into LN containers and thawing was carried out by placing bags removed from LN directly into a 40 °C bath. Viability was evaluated by seed germination tests carried out at 30 °C, in the presence of light.

PERSPECTIVES

The results are promising to the definition of the long term conservation protocol for *Coffea* genetic resources. A core collection of *C. arabica* germplasm is being organized and will be preserved in liquid nitrogen. The protocol is now being adapted to other species of *Coffea*.

ACKNOWLEDGEMENTS

This research was supported by the Brazilian Consortium for Research of Development of Coffee.

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