ZYGOTIC AND SOMATIC EMBRYO CRYOPRESERVATION IN COFFEE (*Coffea arabica, C. canephora* and Arabusta)

A. ABDELNOUR [1], F. ENGELMANN [2], C. HIBJAN [1], V. VILLALOBOS [1], D. GRAY [3], M. DUFOUR [4]

[1] CATIE, 7170 Turrialba, Costa Rica ; [2] ORSTOM, B.P. 5045, Montpellier, France ; [3] IFAS, Univ. of Florida, Leesburg, Florida, USA ; [4] CIRAD-CP/CATIE, 7170 Turrialba, Costa Rica

Abstract

Coffee can successfully be conserved in the field but this method is very expensive and presents high risks of loosing some material through biotic or abiotic events. Therefore we investigated the possibilities of conserving zygotic and clonal (somatic) embryos in liquid nitrogen (LN; at a temperature of -196°C).

First, several pretreatments designed to avoid chilling injury were evaluate on both zygotic and somatic embryos of *C. arabica*, var. catimor and *C. canephora* var. Robusta. Those genotypes were chosen due to their responsiveness in producing embryogenesis. Cultures were initiated by placing 1.5 cm^2 leaf sections on induction medium. Somatic embryos appeared after 10 weeks.

For example, prolonged exposure to high sucrose levels caused the embryos to develop more normally after the cold treatment.

Three cryopreservation protocols were tested.

Treatments included either rapid freezing by direct immersion in LN or slow freezing at either 0.5, 0.8 or 1°C/mn to -40°C prior to immersion in LN. Samples were either slow-thawed for 30 mn or rapid-thawed in a 40°C water bath.

Results from some of these experiments are very promissing. The embryos survived freezing but did not germinate directly. Instead, they produced embryogenic calli and somatic embryos. Robusta embryos showed the higher percentage of survival (71%).

Résumé :

Les caféiers peuvent facilement être conservés sous forme de collections au champ, mais cette méthode est onéreuse et représente des risques élevés de perte de matériel par cause biotique ou abiotique. Nous avons donc recherché la possibilité de conserver des embryons zygotiques et clonaux (somatiques) dans de l'azote liquide ("LN"; à une température de -196°c).

Tout d'abord, plusieurs prétraitements destinés à éviter les dégats dûs au froid ont été essayés à la fois sur les embryons somatiques et zygotiques de *C. arabica* var. catimor et *C. canephora* var. Robusta. Ces génotypes ont été choisis pour leur bonne capacité embryogène. Les cultures sont initiées en plaçant des sections foliaires de 1.5 cm^2 sur un milieu d'induction. Les embryons somatiques apparaissent après 10 semaines. Par exemple, une culture

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prolongée sur de fortes concentrations en saccharose a permis un meilleur développement des embryons.
Trois protocoles de cryoconservation ont été essayés. Les traitements ont consisté en, d'une part, une congélation rapide par immersion directe dans LN, ou, d'autre part en une congélation lente à des rythmes de 0.5, 0.8 ou 1°c/mn jusqu'à -40°c avant immersion dans LN. Les échantillons ont été soit décongelés lentement pendant 30 mn, soit rapidement par trempage dans un bain-marie à 40°c. Les résultats de certaines de ces expériences sont très prometteurs. Les embryons ont survécu à la congélation, mais n'ont pas germé directement. Au contraire, ils produisent des cals embryogènes, puis des embryons somatiques. Les embryons de Robusta ont montré le meilleur pourcentage de survie (71%).

INTRODUCTION

Coffee can successfully be conserved in the field but this method is very expensive and presents high risks of loosing some material through biotic or abiotic events. Therefore we investigated the possibilities of conserving zygotic and clonal (somatic) embryos in liquid nitrogen (LN; at a temperature of -196°C).

MATERIAL AND METHODS

For somatic embryos, two genotypes were used : *Coffea arabica* var Catimor and *Coffea canephora* var Robusta. All the embryogenic cultures were started from leaves from microcuttings cultivated on a standard multiplication medium. Cultures were initiated by placing 1.5 cm^2 leaf sections on induction medium (Yasuda *et al*, 1985). Somatic embryos appeared after 10 weeks.

Three cryopreservation protocols were tested: 1) the protocol reported by Bertrand-Desbrunais *et al*,(1988) which utilizes a culture pretreatment on increasing concentrations of sucrose followed by infiltration with DMSO; a protocol (Dereuddre *et al*, 1990) in which embryos are first placed in alginate beads prior to dehydration in air; 3) finally, a protocol developped at CATIE (Abdelnour-Esquivel *et al*, 1992) involving an air-dehydration pretreatment followed by a rapid freezing in liquid nitrogen and a rapid thawing. Treatments included either rapid freezing by direct immersion in LN or slow freezing at either 0.5, 0.8 or 1°C/min to -40°C prior to immersion in LN. Samples were either slow-thawed for 30 min or rapid-thawed in a 40°C water bath.
Preliminary experiments were also conducted to observe the effects of cryoprotectants such as sucrose (up to 0.75M), DMSO (5, 10 and 15%) and others, on germination of the embryos.

Regarding zygotic embryos, we experimented three different genotypes : *C. arabica* var Caturra, *C. canephora*, and the hybrid arabusta (*C. arabica* x *C. canephora*). Three different stages of maturity were used: embryos from green fruits (ca. two months before harvest), from yellowing fruits (ca. four days before full maturity) and from red mature fruits. The freezing protocol has been previously described (Abdelnour-Esquivel *et al*, 1992).

RESULTS

Zygotic embryos. Most of the results have been previously reported. We observed a 100% germination in the untreated control, and the rate of survival with or without a LN treatment varied according to the moisture content. We also observed a large genotypic effect; *C. canephora* presented a significantly lower survival rate after freezing. Gemination of surviving embryos occured and fully developped plantlets were obtained with all species.

Somatic embryos. Somatic embryos have been obtained directly from leaf discs, with very little or no callus formation. We have been successful in cryopreserving somatic embryos of *C. canephora* but not of *C. arabica*. The first signs of growth appeared between the 5th and the 7th week after freezing. With three replications of the same experiment, it is obvious that there is a large difference between replications. The regrowth percentage at 15 weeks is 72% in one replication and 36% in the other two. Another important difference in response between treatments is the speed of regrowth. Direct immersion into LN was the worst treatment. Embryos pretreated with partial dehydration showed the most rapid regrowth.
We found that the factors that affect success in cryopreservation, as measured by regrowth percentage are : 1) somatic embryo quality and 2) variations in the cooling process inherent to the device used.

DISCUSSION - CONCLUSIONS

For zygotic embryos, we successfully cryopreserved material from *C.arabica, C. canephora* and from the interspecific hybrid arabusta. Plantlets developed directly from those frozen embryos. A simple procedure was used, involving partial desiccation and direct immersion in liquid nitrogen.

For somatic embryos, lack of success with *C. arabica* may be due to the inadequacy of both the somatic embryogenesis medium and the recuperation medium. During the process of somatic embryogenesis, many abnormal embryos are produced from this line and the germination percentage is very low. There is also some evidence of vitrification, a physiological disorder.

Several other trials are under evaluation. We are re-evaluating some embryogenic culture protocols as well as several *C. arabica* genotypes reactivity.

It should therefore be possible to have access to long-term preservation of coffee genetic resources through cryopreservation.

LITERATURE

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Association Scientifique Internationale du Café
(ASIC)
42, rue Scheffer, 75116 Paris