

# CRYOPRESERVATION OF APICES OF *COFFEA RACEMOSA* and *COFFEA SESSILIFLORA* USING THE ENCAPSULATION/DEHYDRATION TECHNIQUE

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## INTRODUCTION

The present study aimed at setting up a cryopreservation technique for apices sampled on *in vitro* plantlets. Apices appeared as the best candidates for long-term preservation of coffee genetic resources. Indeed, genetic characteristics of each species are preserved since plants are multiplied *in vitro* by microcuttings. Moreover, direct growth recovery of apices after cryopreservation is a guarantee for the trueness to type of regenerated plants.

Cryopreservation experiments were performed using the encapsulation-dehydration technique. Indeed, encapsulation-dehydration appears as the only freezing technique likely to ensure both high survival rates and reproducible results in the case of apices (Dereuddre, 1992).

## MATERIALS AND METHODS

### *Plant material*

This study was carried out with *in vitro* plantlets of two coffee species originating from eastern Africa : *Coffea racemosa* and *C. sessiliflora*.

### *Micropropagation*

Plantlets were cultured on an MS solid medium supplemented with sucrose (40 g.l<sup>-1</sup>) and benzyl amino-purine (BAP, 0.3 mg.l<sup>-1</sup>), according to Bertrand-Desbrunais (1991). They were maintained at 27 ± 1°C under a light intensity of 50 µE.m<sup>-2</sup>.s<sup>-1</sup> PAR, with a photoperiod of 12 hrs light / 12 hrs dark.

### *Production and excision of shoot tips*

Plantlets were cut into stem segments comprizing two nodes. They were cultured for three weeks on the same basal medium supplemented with BAP (0.5 mg.l<sup>-1</sup>) and naphtalene acetic acid (NAA, 0.2 mg.l<sup>-1</sup>) in order to induce the development of axillary shoot tips. One mm long shoot tips comprizing 2 to 4 leaf primordia were then excised and placed for recovery in Petri dishes on the same medium, for 24 hrs.

### *Encapsulation*

Shoot tips were suspended in liquid medium devoid of calcium and supplemented with alginic acid (30 g.l<sup>-1</sup>). Encapsulation of apices was achieved by dropping this solution into a liquid medium supplemented with CaCl<sub>2</sub> (100mM), for alginate polymerization.

### *Pregrowth and dehydration*

Alginate beads, containing 1 to 3 shoot tips, were placed for 1 to 10 days in liquid medium supplemented with various sucrose concentrations. In some experiments, beads were transferred daily in media with increasing sucrose concentrations. After preculture, alginate beads were dehydrated for various periods (0 to 6 hrs) in the air current of a laminar flow cabinet.

**Freezing and thawing**

Beads were frozen rapidly by direct immersion in liquid nitrogen (-196°C) where they were kept for a minimum of 20 minutes. They were rewarmed slowly by placing them at room temperature under the laminar flow for 2-3 minutes.

**Recovery**

Encapsulated shoot tips were then placed in Petri dishes containing solid medium. Survival was estimated after 3 to 4 weeks. Apices were considered surviving when leaf expansion and/or growing and callusing was noted. Apices showing signs of recovery were extracted from the beads and placed on the standard medium (BAP 0.3 mg.l<sup>-1</sup>).

**RESULTS**

For both species, survival was generally high for sucrose concentrations comprised between 0.1 and 0.75 M, whatever the pregrowth duration (Table 1). When preculture was performed with 1 M sucrose, survival dropped whatever the pregrowth duration. In all conditions growth recovery of shoot tips was much more rapid with *C. racemosa* than with *C. sessiliflora*. With both species, increasing sucrose concentrations and pregrowth durations considerably slowed down growth recovery.

**Table 1:** Effect of sucrose concentration and pregrowth duration on the survival of shoot tips of *C. racemosa* and *C. sessiliflora*.

	<i>C. racemosa</i>					<i>C. sessiliflora</i>				
	1 day	3 days	5 days	7 days	10 days	1 day	3 days	5 days	7 days	10 days
0.1 M	17/17	14/15	15/15	12/15	12/15	15/15	15/15	15/15	14/15	15/15
0.3 M	17/17	13/15	14/15	8/15	12/15	15/15	15/15	15/15	15/15	15/15
0.5 M	17/17	14/15	10/15	12/15	13/15	15/15	15/15	15/15	14/15	15/15
0.75 M	17/17	11/15	9/15	6/15	13/15	15/15	15/15	12/15	11/15	15/15
1 M	13/17	7/15	7/15	6/15	4/15	6/15	14/15	8/15	7/15	6/15

In all pregrowth conditions experimented, survival of control shoot tips decreased in line with increasing desiccation periods (Table 2). However, dehydration appeared to be less damaging after a 10-day pregrowth period.

Survival of cryopreserved apices was obtained with both species after different pregrowth and desiccation periods. After desiccation, growth recovery of some surviving control and cryopreserved apices occurred in the form of callusing only, whereas other apices turned green and developed directly into plantlets. Cryopreservation considerably slowed down the growth recovery intensity of surviving shoot tips, which could be observed 3-4 weeks after thawing only.

Progressive increase of sucrose concentration during pregrowth did not modify the survival rate of cryopreserved apices of *C. racemosa*. However, it allowed to considerably reduce the delay in growth recovery of cryopreserved apices.

**Table 2:** Effect of preculture conditions and dehydration duration on the survival of control (-LN) and cryopreserved (+LN) shoot tips of *C. racemosa* and *C. sessiliflora*.

	<i>C. racemosa</i>								<i>C. sessiliflora</i>							
	3 days				10 days				3 days				10 days			
	0.5 M		0.75 M		0.5 M		0.75 M		0.5 M		0.75 M		0.5 M		0.75 M	
	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN	- LN	+ LN
Dehydration																
0 h	10/10	0/10	10/10	0/8	8/8	0/7	7/7	0/8	7/7	0/8	7/7	0/8	7/7	0/8	7/7	0/8
1 h 30	10/10	0/10	10/10	0/8	8/8	0/7	7/7	0/8	6/7	0/8	5/7	0/8	7/7	0/8	7/7	0/8
3 h	8/10	0/10	7/10	0/8	8/8	0/7	6/7	0/8	6/7	0/8	3/7	0/8	5/7	0/8	7/7	1/8
4 h 30	3/10	1/10	8/10	1/8	6/8	0/7	2/7	0/8	3/7	0/8	3/7	0/8	3/7	0/8	7/7	1/8
6 h	2/10	2/10	4/10	0/8	6/8	0/7	3/7	0/8	1/8	0/8	4/7	1/8	3/7	0/8	5/7	2/8

**CONCLUSION**

This preliminary study demonstrated that cryopreservation of shoot tips of two coffee species, *C. racemosa* and *C. sessiliflora*, is possible using the encapsulation/dehydration technique. However, additional experiments are necessary in order to increase the survival rates and to improve the recovery pattern of apices. Higher survival rates may be obtained by modifying some parameters of the process. Callusing of apices may be limited or suppressed by refining the hormonal balance of the recovery medium. An histological study is presently carried out in order to observe the damages caused to apices by the successive steps of the cryopreservation process. In a second step the improved process should be experimented with other coffee species. In conclusion, cryopreservation of coffee genetic resources may be foreseeable in a not too distant future using encapsulation/dehydration of apices.

**REFERENCES**

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#### SUMMARY

As regards seed storage behaviour, coffee is classified as recalcitrant since seeds remain viable for very limited periods only. Genetic resources are thus conserved as field collections. *In vitro* collections are being set up which will allow medium-term conservation of germplasm. For long-term storage, cryopreservation (liquid nitrogen,  $-196^{\circ}\text{C}$ ) is the only method currently available. In the case of apices, cryopreservation experiments were performed using the encapsulation/dehydration technique.

Apices sampled on *in vitro* plantlets of *Coffea racemosa* and *C. sessiliflora* were encapsulated in alginate beads, precultured for various durations in liquid medium with high sucrose concentration, partially desiccated under the laminar flow and immersed rapidly in liquid nitrogen. Survival of cryopreserved apices was noted after pregrowth and desiccation periods.

These preliminary experiments indicate that cryopreservation of apices of coffee is possible. Optimal conditions for each step of the process are being sought. They will then be experimented with various coffee species.

#### RESUME

Pour la conservation des graines, le caféier est classifié comme récalcitrant puisque les semences de cette espèce ne restent viables que pendant des durées très limitées. Les ressources génétiques de cette espèce sont donc conservées sous forme de collections en champ. Des vitrothèques sont en cours de mise en place afin de permettre leur conservation à moyen terme. Pour la conservation à long terme, la cryoconservation (azote liquide,  $-196^{\circ}\text{C}$ ) est la seule méthode disponible actuellement. Avec les apex, les essais ont été réalisés en utilisant la méthode d'encapsulation/déshydratation.

Des apex prélevés sur des vitroplants de *Coffea racemosa* ont été encapsulés dans des billes d'alginate, prétraités en milieu liquide à forte concentration en saccharose pendant des durées variables, partiellement déshydratés puis immergés rapidement dans l'azote liquide. La survie d'apex cryoconservés a été obtenue pour différentes durées de prétraitement et de dessiccation.

Ces essais préliminaires indiquent que la cryoconservation d'apex de caféiers est possible. Les conditions optimales de chaque étape du procédé sont actuellement recherchées. Elles seront ensuite expérimentées avec d'autres espèces de caféiers



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