



## ➤ CRYOPRESERVATION IN FRANCE An overview...

First meeting of the ECPGR Working Group « Cryopreservation »  
3-4 May, 2023, Crop Research Institute, PRAG,



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# ➤ CRYOPRESERVATION IN France, an overview...

## 1-Introduction

## 2- Cryopreservation efforts

### A- Seed cryobanking

- *Brassica* cryobank
- *Coffea* cryobank
- *Citrus*

### B- Vegetatively propagated species cryopreservation

- Potato
- *Malus/Pyrus*
- *Hevea/Coffee*

### C-Cryotherapy

- Potato
- Other species (*Pelargonium, Ananas, Dioscorea sp, Vanilla, Vitis* )

### D-Forest species

- International *Ulmus* cryobank
- Cryopreservation of pine clones as embryogenic cultures

## 3 Prospects and Perspectives

## > Seed Cryobanking

### ❖ *Brassica* cryobank

Orthodox seeds with high lipid content >>>>>>>> population regeneration in 5-year cycles

Presently, 1016 *Brassica* populations

- 907 *B. oleracea*,
- 71 *B. napus*
- 38 *B. rapa*).

conserved in liquid nitrogen as five tubes of 250 seeds each.

Aims: safety duplicates/ assessment of possible genetic shifts after a number of multiplication cycles

# > Cryobanking

## ❖ *Coffea* cryobank

Intermediate seeds

>>>>>>cryopreservation after dehydration

Presently, 337 *Coffea* accessions from the Réunion Island collection have been cryopreserved, i.e. 62% of the accessions.

Cryobanking is now to resume after a technician has been contracted for the next two years (fresh seed collection, conditioning and shipping)

As of today, the first cryobank of intermediate seeds

Species	Number of accessions currently in the collection and how they are stored				Material cryopreserved
	Total	Field	In cryo	% of cryopreserved accessions	
<i>Coffea anthonyi</i>	25	25	20	<b>80%</b>	seeds
<i>Coffea arabica</i>	178	98	128	<b>72 %</b>	seeds
<i>Coffea costatifructa</i>	9	9	9	<b>100%</b>	seeds
<i>Coffea eugenioïdes</i>	13	13	13	<b>100%</b>	seeds
<i>Coffea heterocalyx</i>	4	4	1		seeds
<i>Coffea myrtifolia</i>	15	15	1		seeds
<i>Coffea pocsii</i>	10	10	9	<b>90%</b>	seeds
<i>Coffea pseudozan.</i>	16	16	16	<b>100%</b>	seeds
<i>Coffea racemosa</i>	21	21	21	<b>100%</b>	seeds
<i>Coffea salvatrix</i>	14	14	12		seeds
<i>Coffea sessiliflora</i>	10	10	10	<b>100%</b>	seeds
<i>Coffea sp. Congo</i>	9	9	4		seeds
<i>Coffea stenophylla</i>	20	20	14	<b>70%</b>	seeds
<i>Coffea brevipes</i>	18	18	12	<b>67%</b>	embryos
<i>Coffea canephora</i>	84	84	12		embryos
<i>Coffea congensis</i>	25	25	22	<b>88%</b>	embryos
<i>Coffea dewevrei</i>	40	40	21	<b>50%</b>	embryos
<i>Coffea humilis</i>	11	11	2		embryos
<i>Coffea liberica</i>	23	23	10		embryos
<b>TOTAL</b>	<b>545</b>		<b>337</b>	<b>62%</b>	

## > Cryobanking

- ❖ Citrus cryobanking (starts soon...)

Intermediate seeds, highly heterozygous species, BUT

presence of nucellar embryos (70% of all seed-bearing accessions)

>>>>>> the original genetic combination can be maintained through seed cryobanking

Presently, preliminary experiments have shown differences in sensitivity to dehydration, with *Poncirus* species being highly sensitive while lemons and limes are more tolerant.

Cryobanking is to start very soon in Corsica as most technical problems have been solved (not. LN production locally)

Problems for desiccation-sensitive, seedless varieties and those bearing only monoembryonic seeds

>>>> apex cryopreservation (USDA, Fort Collins)

# ➤ Cryopreservation of vegetatively multiplied species

## ❖ Potatoes and allied species cryobanking

Mainly *Solanum tuberosum* (78 clones) and 25 other related species conserved after PVS2 treatment and droplet vitrification.

At present, 152 clones have « validated » cryopreserved samples (i.e. with a regeneration rate over 40%) with 100 apices per clone.



Species	Number of cryopreserved clones
<b>S.tuberosum</b>	<b>78</b>
S.acaule	1
S.andreanum	1
S.alandiae	8
S.albicans	2
S.berthaultii	1
S.brachistotrichum	4
S.bulbocastanum	3
S.cardiophyllum	3
S.chacoense	1
S.chaucha	1
S.commersonii	2
S.demissum	1
S.fendleri	5
S.kurtzianum	1
S.oplocense	3
S.phureja	1
<b>S.polyadenium</b>	<b>14</b>
S.polytrichon	6
S.spegazzinii	1
S.stenotomum	1
S.stoloniferum	7
S.tarijense	1
S.trifidum	4
S.vernei	1
Unknown species (69. 56. 52)	1
	<b>152</b>

## ➤ Cryopreservation of vegetatively multiplied species

### ❖ *Malus/Pyrus* cryobanking

2011-2015: Improvement of protocols: dormant bud technique and droplet vitrification.

As of 2023, 193 *Malus* accessions preserved :

- 2019: 16
- 2020: 18
- 2021: 45
- 2022: 36
- 2023: 36

Development of a vitrification technique for Pear, no cryobanking as such....

Arnaud Guyader, Agnès Grapin, Camille Le Bras, François Laurens. Development of the droplet-vitrification cryopreservation technique as a viable alternative to the dormant bud technique for recalcitrant pears. 26-28 mars 2018 Bangkok Thailand. Acta Hort. 1234, 219-224, DOI:0.17660/ActaHortic.2019.1234.29



## ➤ Cryopreservation of vegetatively multiplied species

### ❖ Other species

#### *Hevea/Coffea* embryogenic cultures

- *Hevea*: cryopreservation (in sucrose/DMSO solution) with slow cooling of single friable embryogenic calli either as
  - Back-up of embryogenic lines (3-5 cryotubes)
  - Amplification of the few initial calli (20-30 cryotubes)
  - Selection of vigorous embryogenic lines for field testing and genetic transformation (up to 100 cryotubes maintained)
  - Cryopreservation of transformed embryogenic lines
  
- *Coffea*: cryopreservation with slow cooling of embryogenic lines to be used for genetic transformation

## ➤ Cryotherapy in vegetatively multiplied species

- ❖ *Pelargonium*: previous thesis project, no current activity...
- ❖ **On-going projects (funding by GIS IBiSA: initial petition 149 k€ >>>> granted 40 k€ !?)**
  - *Solanum* species: 18 accessions with various viruses alone or in combination
  - *Ananas* species : three species regenerated after PVS2-based vitrification...to be PCR-tested for presence of viruses
  - Project Yam and *Vanilla*
  - *Vitis vinifera*: sanitation actions linked to the transfer of the French grapevine repository: 3000-3500 accessions to be sanitized, aim to use at least in part cryotherapy.

## ➤ Conclusions and perspectives

- ❖ Several sizeable cryobanks have already been implemented, viz. *Brassica*, *Coffea*, *Malus*, *Solanum*, forest species)
- ❖ Other cryopreservation applications are routinely used (*Hevea*, *Coffea*)
- ❖ BRC in care of vegetatively propagated species are all interested in safety duplicates/back-ups (whatever the technique) (survey S. DUSSERT)
- ❖ The same are also very interested in improved sanitation methods (also whatever the technique)

### Perspectives

- ❖ Most of these BRC do not have trained personnel and/or facilities for implementing safety duplicates/sanitation methods
- ❖ Long-term efforts for cryopreservation implementation are difficult to envision (staff number maintained at best, no facilities...)
- ❖ Cryotherapy implementation seems to deliver more immediate results with direct effect on resources centres main missions, accession preservation, improved dissemination