

Spanish Cryopreservation

1st Meeting of the ECPGR Cryopreservation Working Group

3-4 May 2023

Crop Research Institute, Prague, Czech Republic



Spanish representatives

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Cryopreservation expert

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Genebank curator

Conchi Sánchez Fernández

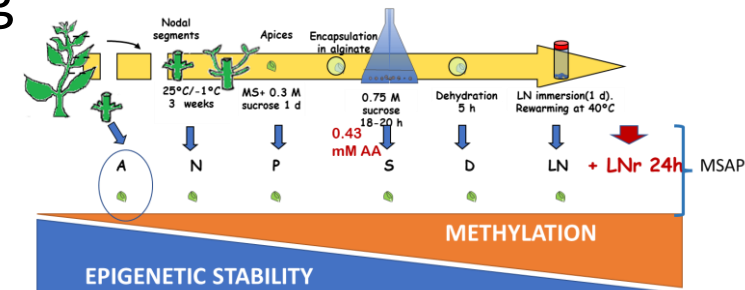
Misión Biológica de Galicia (MBG)-CSIC
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Researcher, crop specialist
(oak, chestnut and cork oak)

What do we do

- M. Elena Gonzalez Benito
 - Epi-genetic stability analysis after cryopreservation (shoot tips, vitrification and encapsulation-dehydration)
- Conchi Sanchez Fernandez
 - Cryopreservation by vitrification (shoot apex and somatic embryos) and genetic stability analysis.
- Mayte Espiau Ramírez
 - Fruit tree germplasm bank curator, using in vitro conservation and aiming to use cryopreservation for long term storage



Research projects

2015	OXIDATIVE DAMAGE AND EPI-GENETIC STABILITY AFTER CRYOPRESERVATION (GARLIC, MINT)*	UNIVERSIDAD POLITECNICA DE MADRID	MADRID
2016	CRYOPRESERVATION OF EMBRYOGENIC IN VITRO CULTURES OF QUERCUS SUBER AND Q. ILEX*	AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (CSIC)	A CORUÑA
2021	CRYOPRESERVATION OF EMBRYOGENIC CELL CULTURES OF MARINE PLANTS (<i>POSIDONIA OCEANICA</i> AND <i>CYMODOCEA NODOSA</i>)*	UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA	LAS PALMAS

* Partial objective

Cryopreserved collections

- TRAGSA (Galicia)
 - Cork oak: cryoprotected somatic embryos by vitrification stored at -150°C (180 genotypes)
 - Chestnut : cryoprotected shoot apex by vitrification stored at -150°C (130 clones of chestnut hybrids and *C. sativa*).
- NEIKER (Basque Country)
 - Pinus: approx. 500 lines of cryoprotected embryogenic cultures by vitrification, stored at -80°C (economic reasons)
 - Solanum: previous cryopreserved collection (60 accessions) not maintained due to economic reasons
- MBG (Galicia)
 - Oak: cryopreserved somatic embryos by vitrification (6 genotypes)
 - Oak: cryopreserved transformed somatic embryos by vitrification (21 lines from 4 genotypes)
 - Chestnut and holm oak: cryopreserved somatic embryos by vitrification.
- IVIA (Valencia)
 - Citrus: 42 embryogenic callus collection

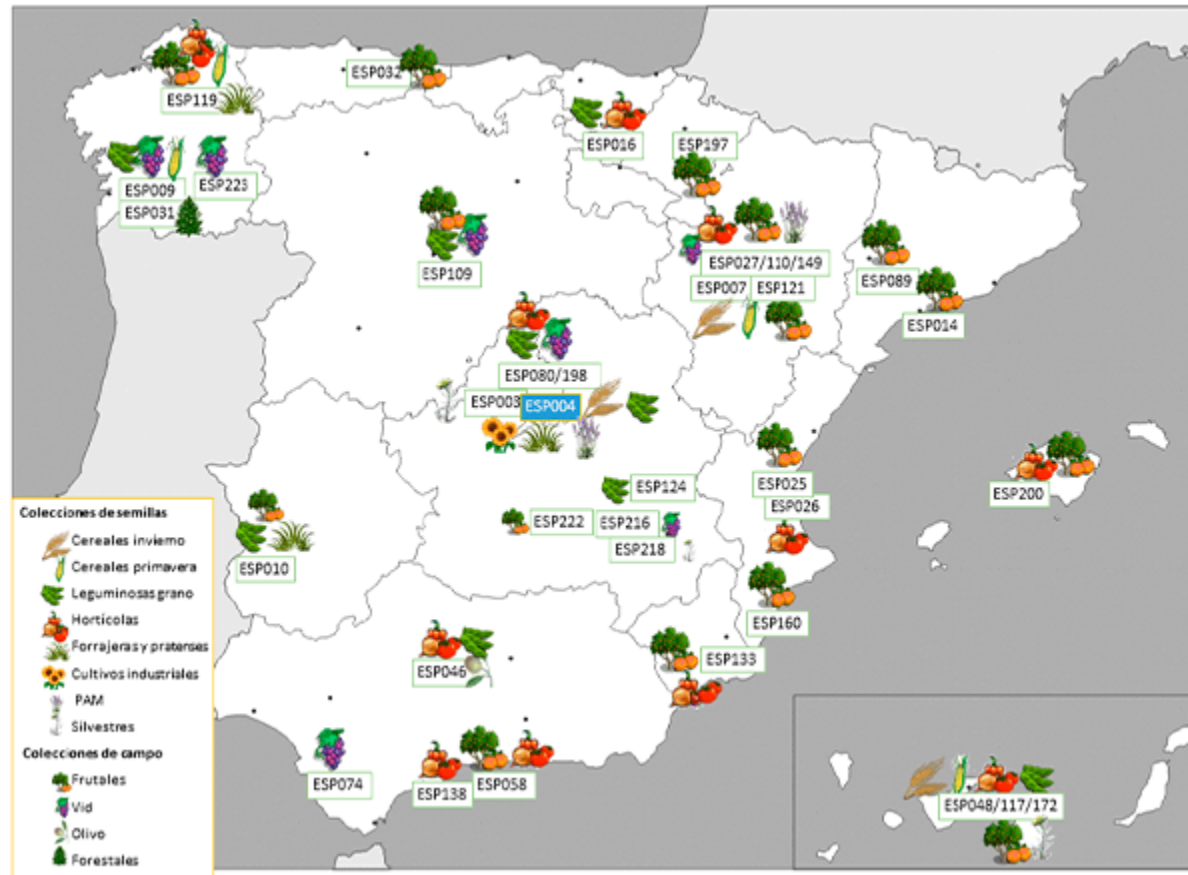


In vitro preservation of shoot cultures at low temperature

- MBG
 - Chestnut: internodes from *in vitro* shoots stored at 4°C (10 genotypes)
 - Oak: internodes and shoot apex from *in vitro* shoots stored at 4°C (20 genotypes)
 - Cor oak: internodes from *in vitro* shoots stored at 4°C
 - Fruit and ornamental trees: internodes and shoot apex from *in vitro* shots stored at 4°C (15 genotypes)
- CULTIGAR (Galicia)
 - Oak: internodes and shoot apex from *in vitro* shoots stored at 4°C (20 genotypes)
 - Fruit and ornamental trees: internodes and shoot apex from *in vitro* shots stored at 4°C (6 genotypes)
- CITA (Aragón)
 - Pear: 32 genotypes of Spanish cultivars stored *in vitro* to be transferred at 1°C this year (new facility)

National Network PGR

Coordinator: Centro Nacional de Recursos Fitogenéticos



PGR Spanish national inventory

Vegetative plant material

Genera	Total number accession	N. germplasm banks
<i>Allium</i>	900	2
<i>Castanea</i>	257	2
<i>Citrus</i>	382	2
<i>Corylus</i>	285	2
<i>Crocus</i>	239	1
<i>Fragaria</i>	165	2
<i>Malus</i>	1823	8
<i>Prunus</i>	1829	8
<i>Pyrus</i>	1162	6
<i>Solanum</i>	264	2
<i>Vitis</i>	5704	11



PGR Spanish national inventory

In vitro collections

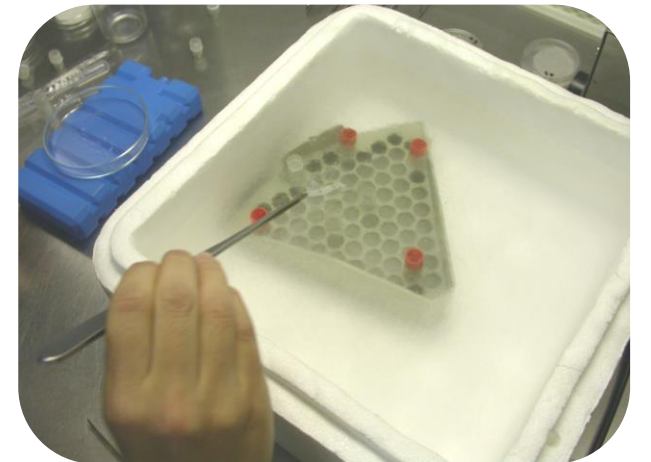
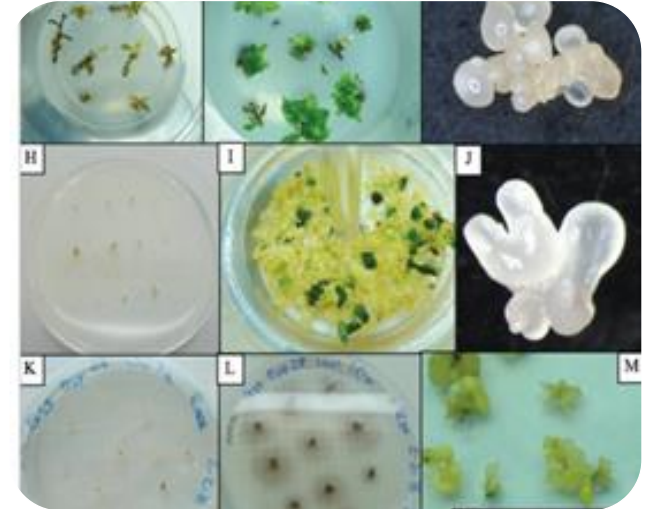
Genera	Total number accession	N. germplasm banks
Solanum	162	1
Pear	32	1



Neiker

Cryobiology

- SECRIO - Spanish Society of Cryobiology
 - Recently created
 - Focused on the study of the effect of low temperatures to plant and animal structures
- On going research work
 - Universidad de Valencia
 - Universidad de Málaga



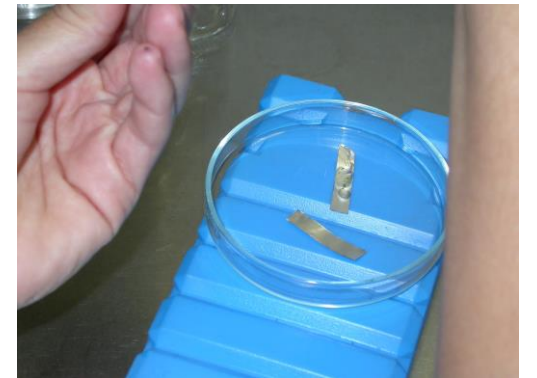
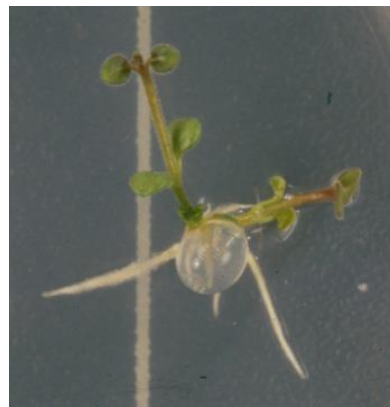
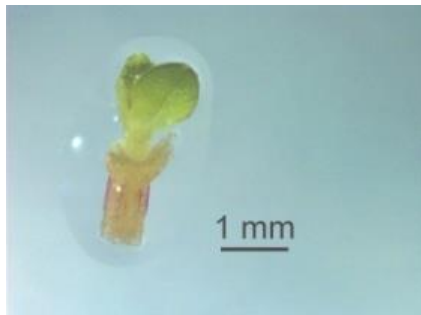
Constraints

- Financial support
 - For PGR *ex situ* conservation (worse for the last five years)
 - No real national strategy for vegetative material
 - For cryopreservation:
 - Scarce support to applied science
 - Scarce support for long-term liquid nitrogen acquisition



Proposals/ideas for a European strategy

- Improve awareness of the Spanish Government for the need of long-term storage of vegetative PGR
- Exchange of protocols and experience
- More trained technicians
- European Project Proposal



A blue rectangular tag with the words "Thank You!" written in white cursive script. The tag is surrounded by a large amount of crushed, clear plastic material, likely representing recycled plastic. A blue cord is attached to the top of the tag, and two clear plastic pieces are attached to the sides of the tag.

Thank
You!