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

Mayte Espiau Ramirez
Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)
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Genebank curator
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

<table>
<thead>
<tr>
<th>Year</th>
<th>Title</th>
<th>Institution</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>OXIDATIVE DAMAGE AND EPI-GENETIC STABILITY AFTER CRYOPRESERVATION (GARLIC, MINT)*</td>
<td>UNIVERSIDAD POLITECNICA DE MADRID</td>
<td>MADRID</td>
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<tr>
<td>2016</td>
<td>CRYOPRESERVATION OF EMBRYOGENIC IN VITRO CULTURES OF QUERCUS SUBER AND Q. ILEX*</td>
<td>AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (CSIC)</td>
<td>A CORUÑA</td>
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<tr>
<td>2021</td>
<td>CRYOPRESERVATION OF EMBRYOGENIC CELL CULTURES OF MARINE PLANTS (POSIDONIA OCEANICA AND CYMODOCEA NODOSA)*</td>
<td>UNIVERSIDAD DE LAS PALMAS DE GRAN CANARIA</td>
<td>LAS PALMAS</td>
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</table>
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 4ºC (10 genotypes)
  • Oak: internodes and shoot apex from *in vitro* shoots stored at 4ºC (20 genotypes)
  • Cor oak: internodes from *in vitro* shoots stored at 4ºC
  • Fruit and ornamental trees: internodes and shoot apex from *in vitro* shoots stored at 4ºC (15 genotypes)

• CULTIGAR (Galicia)
  • Oak: internodes and shoot apex from *in vitro* shoots stored at 4ºC (20 genotypes)
  • Fruit and ornamental trees: internodes and shoot apex from *in vitro* shoots stored at 4ºC (6 genotypes)

• CITA (Aragón)
  • Pear: 32 genotypes of Spanish cultivars stored *in vitro* to be transferred at 1ºC this year (new facility)
National Network PGR
Coordinator: Centro Nacional de Recursos Fitogenéticos
PGR Spanish national inventory
Vegetative plant material

<table>
<thead>
<tr>
<th>Genera</th>
<th>Total number accession</th>
<th>N. germplasm banks</th>
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</thead>
<tbody>
<tr>
<td>Allium</td>
<td>900</td>
<td>2</td>
</tr>
<tr>
<td>Castanea</td>
<td>257</td>
<td>2</td>
</tr>
<tr>
<td>Citrus</td>
<td>382</td>
<td>2</td>
</tr>
<tr>
<td>Corylus</td>
<td>285</td>
<td>2</td>
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<tr>
<td>Crocus</td>
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<td>1</td>
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<tr>
<td>Fragaria</td>
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<td>2</td>
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<tr>
<td>Malus</td>
<td>1823</td>
<td>8</td>
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<td>Prunus</td>
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<tr>
<td>Pyrus</td>
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<td>6</td>
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<tr>
<td>Solanum</td>
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<td>2</td>
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<tr>
<td>Vitis</td>
<td>5704</td>
<td>11</td>
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</table>
PGR Spanish national inventory

In vitro collections

<table>
<thead>
<tr>
<th>Genera</th>
<th>Total number accession</th>
<th>N. germplasm banks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum</td>
<td>162</td>
<td>1</td>
</tr>
<tr>
<td>Pear</td>
<td>32</td>
<td>1</td>
</tr>
</tbody>
</table>
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
Thank You!