The status of cryopreservation in Slovenia

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1st Meeting of the ECPGR Cryopreservation Working Group, 3-4 May 2023 Crop Research Institute, Prague, Czech Republic
Endemic

*Hladnikia pastinacifolia* (Apiaceae)
Cryopreservation, a tool for long-term preservation of endangered species

- a monotypic endemic genus
- unique position within Apiaceae family,
- low genetic variability – RAPD,
- extremely narrow distribution area (4 km$^2$; Trnovski gozd, Slovenia) in one larger and three smaller, isolated populations
- tertiary relic,
- rare and protected by legislation.

*Hladnikia pastinacifolia* Rchb. (Apiaceae)
Cryopreservation, a tool for long-term preservation of endangered species.

**BIODIVERSITY CONSERVATION**

<table>
<thead>
<tr>
<th>In situ</th>
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<tbody>
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Decree on Rare and Threatened Wild Plant Species (Official Gazette of the Republic of Slovenia 2002; 2010), Natura 2000 species
Cryopreservation, a tool for long-term preservation of endangered species.

**BIODIVERSITY CONSERVATION**

*In situ*

1. Protected areas
   - National parks
   - Wildlife sanctuaries
   - Biosphere Reserves
     - Terrestrial
     - Marines

2. Sacred areas
   - lakes, forest, groves

*Ex situ*

1. Conventional
   - Botanical gardens
   - Arboreta
   - Zoological gardens
   - Vivaria (teraria, aquaria)
   - Seed, polen banks
   - Gene banks

2. *In vitro* based
   - Micropropagation
   - Slow growth (low T)
   - Cryopreservation - tissue banking in LN (liquid nitrogen)

Decree on Rare and Threatened Wild Plant Species (Official Gazette of the Republic of Slovenia 2002; 2010), Natura 2000 species
Micropropagation of the narrow endemic *Hladnikia pastinacifolia* (Apiaceae)

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Cryopreservation, a tool for long-term preservation of endangered species
Cryopreservation, a tool for long-term preservation of endangered species

**Precondition of plant material**

T = 4°C in the dark for 7–14 d

**Precultivation of explants**

sucrose, 12–14 h

**Encapsulation/ polymerization**

sucrose/glycerol, 30–60 min

**Encapsulation-dehydration** (ED)

**Encapsulation-vitrification** (EV)

**Droplet vitrification** (DV)

**Osmoprotection**

Liquid MS with sucrose/glycerol, 10–12 h

Liquid MS with sucrose/glycerol, 30 min

**Drying**

Laminar flow or desiccator

**Vitrification**

PVS2, 30 min

**Cryopreservation LN***

Direct** immersion in 2 mL cryovials

Immersion in 2 mL cryovials, with PVS2

Direct** immersion in 6 mL cryovials

*LN, liquid nitrogen

**without cryoprotectant medium
Cryopreservation, a tool for long-term preservation of endangered species

**ED** Encapsulation-dehydration
**EV** Encapsulation-vitrification
**DV** Droplet vitrification

**Cryopreservation LN**
- Direct** immersion in 2 mL cryovials
- Immersion in 2 mL cryovials, with PVS2
- Direct** immersion in 6 mL cryovials

**Rewarming**
- 5 min at 20°C
- 1 min at 40°C
- 5 min at 20°C

**Rehydration**
- Last used cryoprotection solution, 15 min
- Washing (3x) in liquid MS with 1.2 M sucrose
- Unloaded in (3x) liquid MS with 1.2 M sucrose

**Regrowth**
MS (1 wk) and then in MS with 2 µM BAP (2 wk) and later in MS with 5µM BAP and 3 µM IBA

- 53 %
  Ciringer et al. 2018
- 64 %
  Ciringer et al. 2018
- 38 %
  In development!

*LN, liquid nitrogen
**without cryoprotectant medium
Cryopreservation of an endangered Hladnikia pastinacifolia Rchb. by shoot tip encapsulation-dehydration and encapsulation-vitrification

Tereza Ciringer1, Carmen Martin2, Nina Šajna1, Mitja Kaligarič1, Jana Ambrožič-Dolinšek1,3

Precultivation of shoots → Encapsulated shoots → Regrowth

Figure 3. Example of random amplification of polymorphic DNA (RAPD) analysis obtained from control and cryopreserved samples of genotypes L3 and L9 of Hladnikia pastinacifolia using the primers All, E19, OPQ-14. C non-cryopreserved control; ED encapsulation-dehydration; EV encapsulation-vitrification; M molecular weight scale (DNA ladder).
Long-term storage facilities:
Hladnikia pastinacifolia (Apiaceae)

Thank you!

The research was supported by The Slovenian Research Agency program Research for improvement of safe food and health (ARRS, Code P1-0164); and by the project Development of Research Infrastructure for the International Competitiveness of the Slovenian RRI Space—RI-SI-LifeWatch; and by the Republic of Slovenia, Ministry of Education, Science and Sport and the European Union from the European Regional Development Fund.