



Faculty of Natural Sciences
and Mathematics



DEPARTMENT OF BIOLOGY

The status of cryopreservation in Slovenia

Jana Ambrožič-Dolinšek



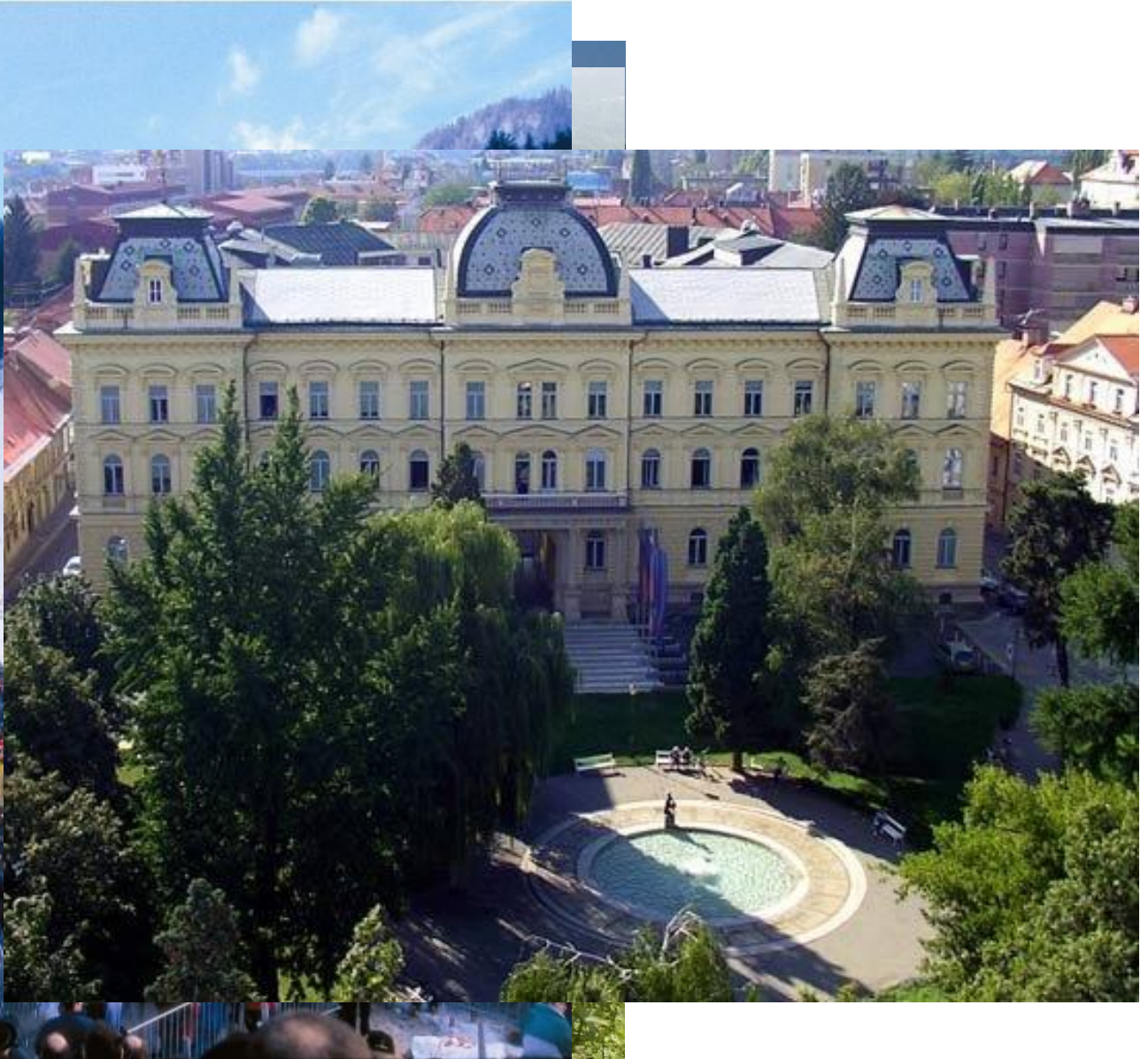
University of Maribor
Faculty of Natural Sciences
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Department of Biology
<https://biologija.fnm.um.si/>



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







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Endemic
***Hladnikia pastinacifolia* (Apiaceae)**





- a monotypic endemic genus
- unique position within Apiaceae family,
- low genetic variability – RAPD,
- extremely narrow distribution area (4 km²; Trnovski gozd, Slovenia) in one larger and three smaller, isolated populations
- tertiary relic,
- rare and protected by legislation.



Hladnikia pastinacifolia Rchb. (Apiaceae)



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, pollen banks
- Gene banks

2. *In vitro* based

- Micropropagation
- Slow growth (low T)
- Cryopreservation - tissue banking in LN (liquid nitrogen)



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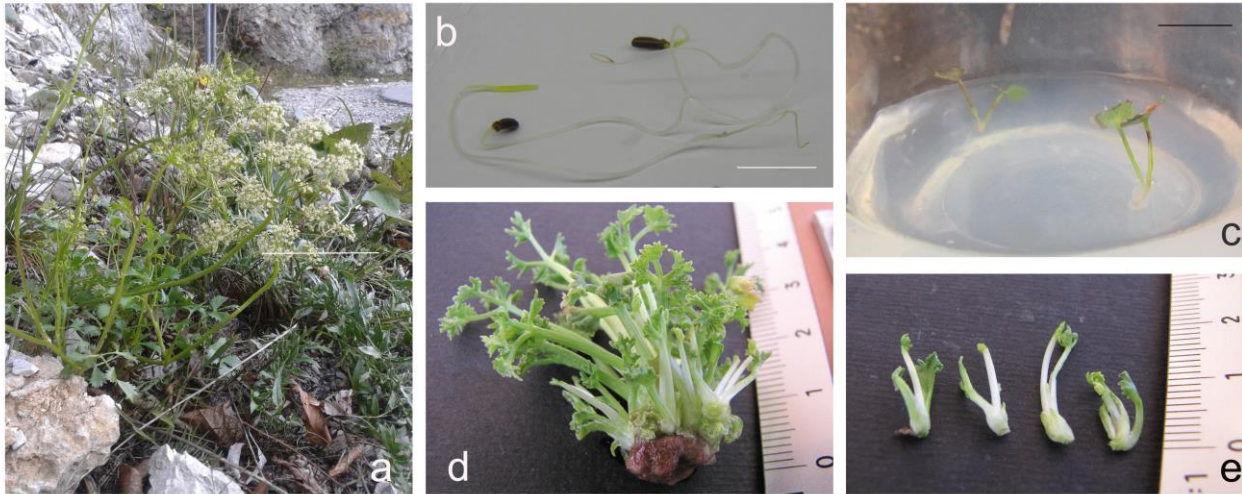
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Micropropagation of the narrow endemic *Hladnikia pastinacifolia* (Apiaceae)

Jana Ambrožič-Dolinšek^{1,2,3*}, Terezija Ciringer¹, Mitja Kaligarič^{1,3}

¹ University of Maribor, Faculty of Natural Sciences and Mathematics, Koroška 160, Maribor, Slovenia

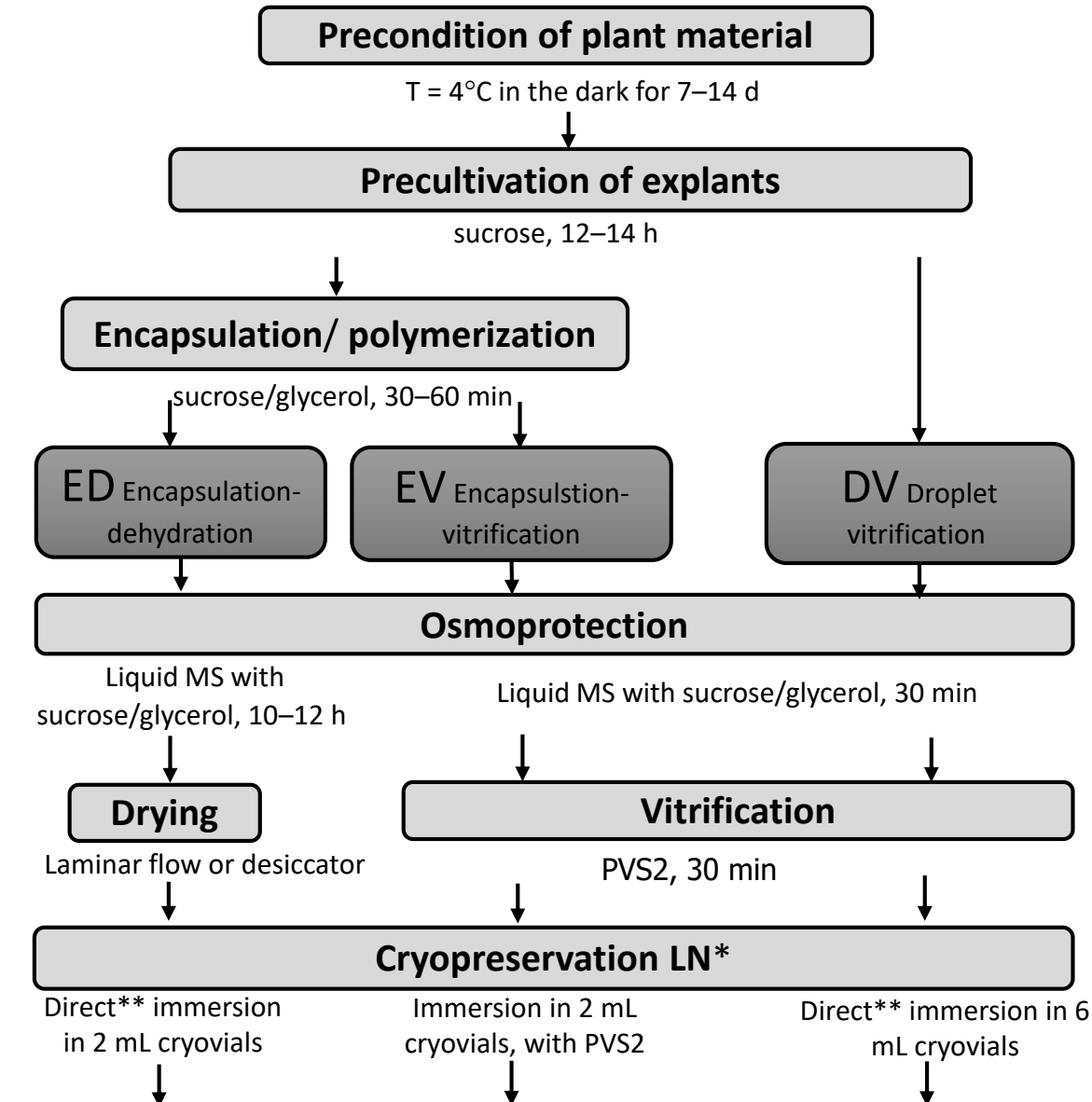
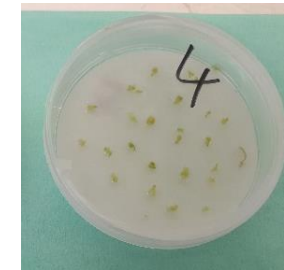
² University of Maribor, Faculty of Education, Koroška 160 Maribor, Slovenia

³ University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, 2311 Hoče, Slovenia



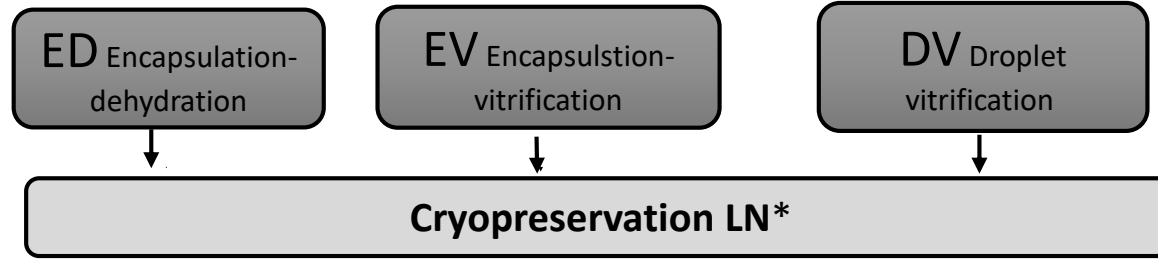
Acta Bot. Croat. 75 (2), 244–252, 2016

DOI: 10.1515/botcro-2016-0028



*LN, liquid nitrogen

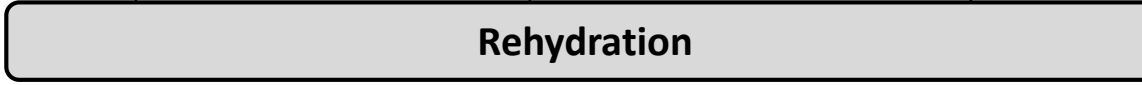
**without cryoprotectant medium



ED Direct** immersion in 2 mL cryovials
EV Immersion in 2 mL cryovials, with PVS2
DV Direct** immersion in 6 mL cryovials



ED: 5 min at 20°C
 EV: 1 min at 40°C
 DV: 5 min at 20°C



ED Last used cryoprotection solution, 15 min
EV Washing (3x) in liquid MS with 1.2 M sucrose
DV Unloaded in (3x) liquid MS with 1.2 M sucrose

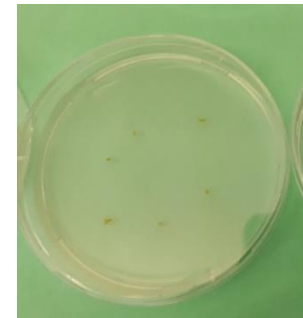
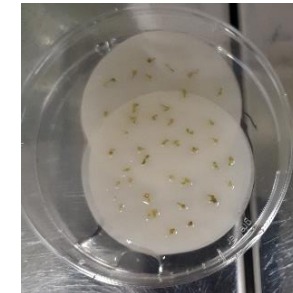
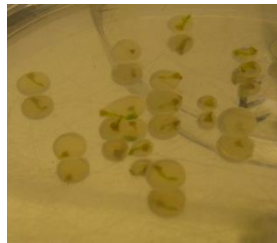


MS (1 wk) and then in MS with 2 μM BAP (2 wk) and later in MS with 5 μM BAP and 3 μM IBA

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

Terezija Ciringer¹ · Carmen Martín² · Nina Šajna¹ · Mitja Kaligarič¹ · Jana Ambrožič-Dolinšek^{1,3}

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Precultivation of shoots → Encapsulated shoots → Regrowth

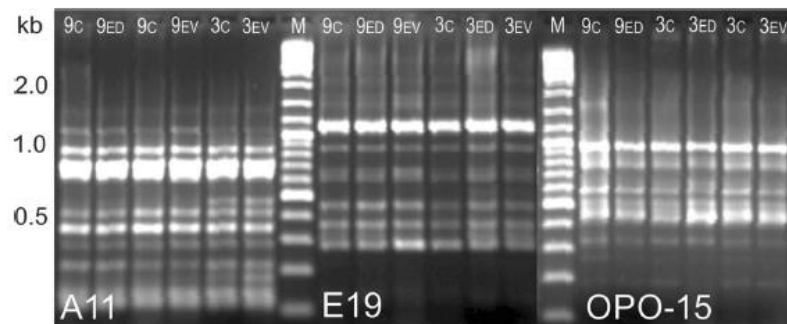
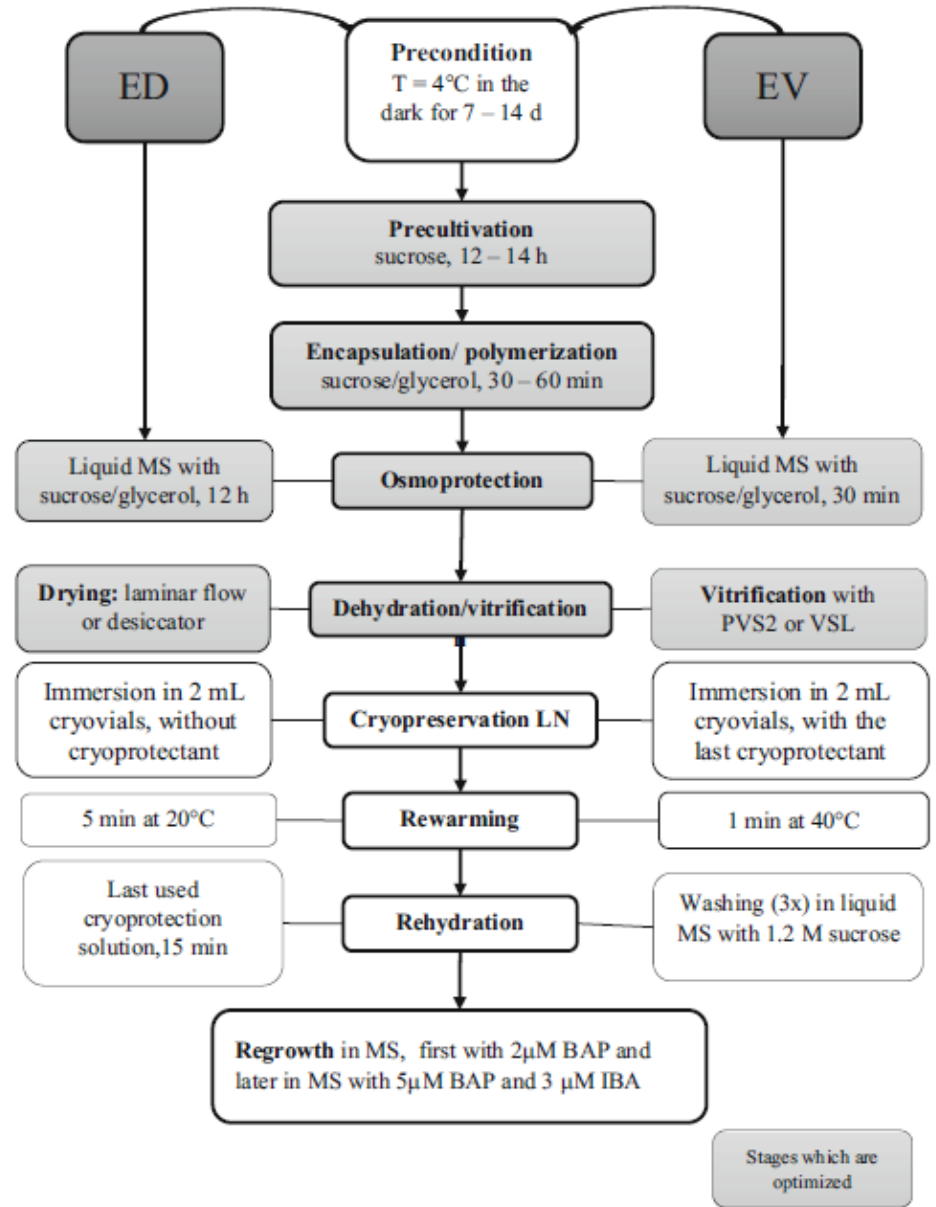


Figure 3. Example of random amplification of polymorphic DNA (RAPD) analysis obtained from control and cryopreserved samples of genotypes *L3* and *L9* of *Hladnikia pastinacifolia* using the primers *A11*, *E19*, *OPQ-14*. *C* non-cryopreserved control; *ED* encapsulation-dehydration; *EV* encapsulation-vitrification; *M* molecular weight scale (DNA ladder).



Long-term storage facilities:





Hladnikia pastinacifolia (Apiaceae)

Thank you!



University of Maribor
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CIRINGER, Terezija, PhD

Carmen MARTÍN, Elena GONZÁLEZ BENITO



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