Cryobanking of Plant Genetic Resources in the Czech Republic

Cryopreservation as Safety Duplication

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1st Meeting of the ECPGR Cryopreservation Working Group

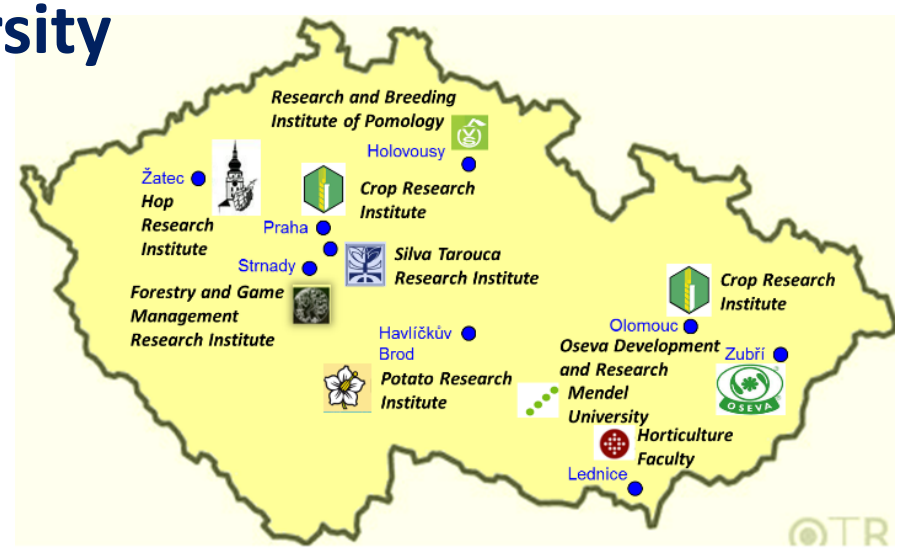
3-4 May 2023, Crop Research Institute, Prague, Czech Republic

The National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity

- **Organized by Ministry of Agriculture**
- **Coordinated by the Crop Research Institute**
- **Board of plant genetic resources - curators of generatively and vegetatively propagated crops**
  - Generatively propagated crops (cereals, ..) stored in form of seed at low temperature for few or tens years in the Central Seed Genebank
  - **Vegetatively propagated crops – storing in form of seeds is not possible, stored in vegetatively propagated part of plants – tubers, bulbs, cuttings, *ex vitro* explants or intact plants in field conditions; backup in the Central Cryobank**

The National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity

Vegetatively propagated crops - National curators:



- Potato research Institute Havlíčkův Brod – potato (*in vitro*)
- Hop Research Institute Žatec – hop
- MENDELU Lednice – thermophilic temperate fluit trees
- CRI Olomouc – *Allium*
- VSV Karlštejn CRI, Ampelos Vrbovec, MENDELU Lednice – *Vitis*
- Research and Breeding Institute of Pomology Holovousy – temperate fruit trees

The National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity

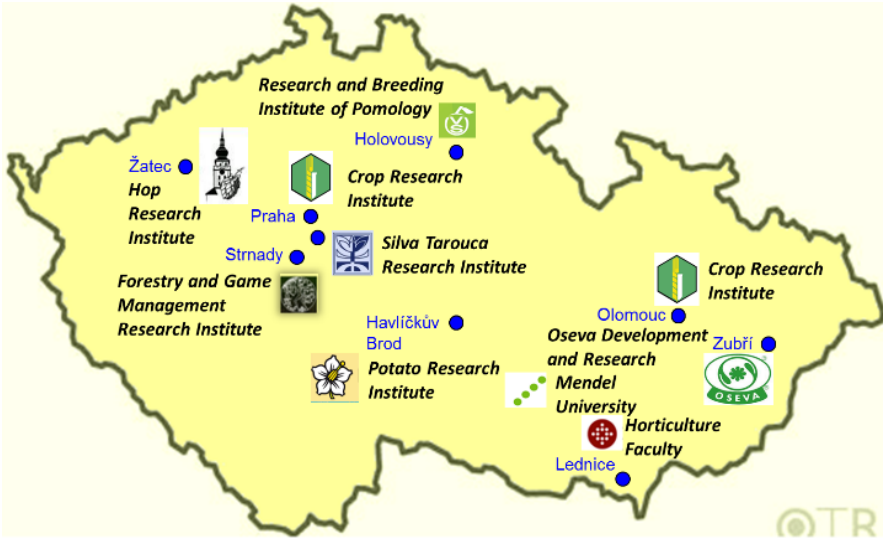
Vegetatively propagated crops - National curators:

Basic strategy of plant germplasm cryoconservation

– safety duplication of basic collections (different storage method and locality)

– storing the most valuable genetic material of the Czech origin

Central cryobank in the frame of „National program“ – collaborates with plant germplasm curators, that provide the most valuable samples for their backup.



Current Cryopreservation Activities

Cryobank - current state

Crop number	Crop code	Crop name	Number of accessions
1	F01	*Malus domestica* BORK	17
2	F07	*Pyrus communis* L. (E	24
3	F24	*Prunus armeniaca* L.	12
4	F28	*Persica vulgaris* P.M	5
5	F35	*Cerasus avium* (L.) M	3
6	F37	*Cerasus vulgaris* P.M	10
7	F38	*Cerasus* P.MILLER (ot	3
8	F46	*Fragaria x ananassa*	34
9	F80	*Lonicera* L. (edible	24
10	H01	*Allium* sativum L.	187
11	S01	*Solanum tuberosum* L1	104
12	V01	*Vitis vinifera* L.	3
13	W93	*Malus* MILL. <hort. c	6
14	X90	*Humulus lupulus* L.	68
Total			500

Current Cryopreservation activities

FUNDING

- Institutional project 22%
- National projects 32%
- International project 37%
- National program 9% (0.7 personal capacity)

Current Cryopreservation activities

Tripartite German-Czech-Polan *Allium* cryobank - preservation of valuable accessions of garlic gene pools on the basis of mutual reciprocity within the framework of tripartite international cooperation, which is the result of a joint **GENRES** research project called **EURALLIVEG** (Jiri Zamecnik)

“ Healthy berries in a changing climate: development of new biotechnological procedures for virus diagnostics, vector studies, elimination and safe preservation of strawberry and raspberry “ – international cooperation project Czech Rep. + Norway (NIBIO) (Alois Bilavcik)

“Nanocomposite hydrogels for cryopreservation of plant genetic resources“ within the programme Horizon Europe, call “MSCA4Ukraine” - Grant Agreement No. 1233650 (Olena Bobrova)

Genotyping-by-sequencing of the European garlic collection to develop a sustainable ex situ conservation strategy (Garli-CCS) - Sixth Call, Phase X, ECPGR Grant

Cryopreservation protocols

- **plant material** – *ex vitro* , *in vitro*
- **acclimation** – low temperature, osmotic
- **methods** - two-step freezing, encapsulation-dehydration, simple-dehydration, vitrification, droplet-vitrification
- **recovery** - safe cryopreservation and recovery of samples

Cryopreservation protocols

- **Two-step freezing – dehydration by freezing**
- **Encapsulation-dehydration – dehydration by dry air**
- **Simple-dehydration – dehydration by dry air**
- **Vitrification – osmotic dehydration**
- **Droplet-vitrification – osmotic dehydration**

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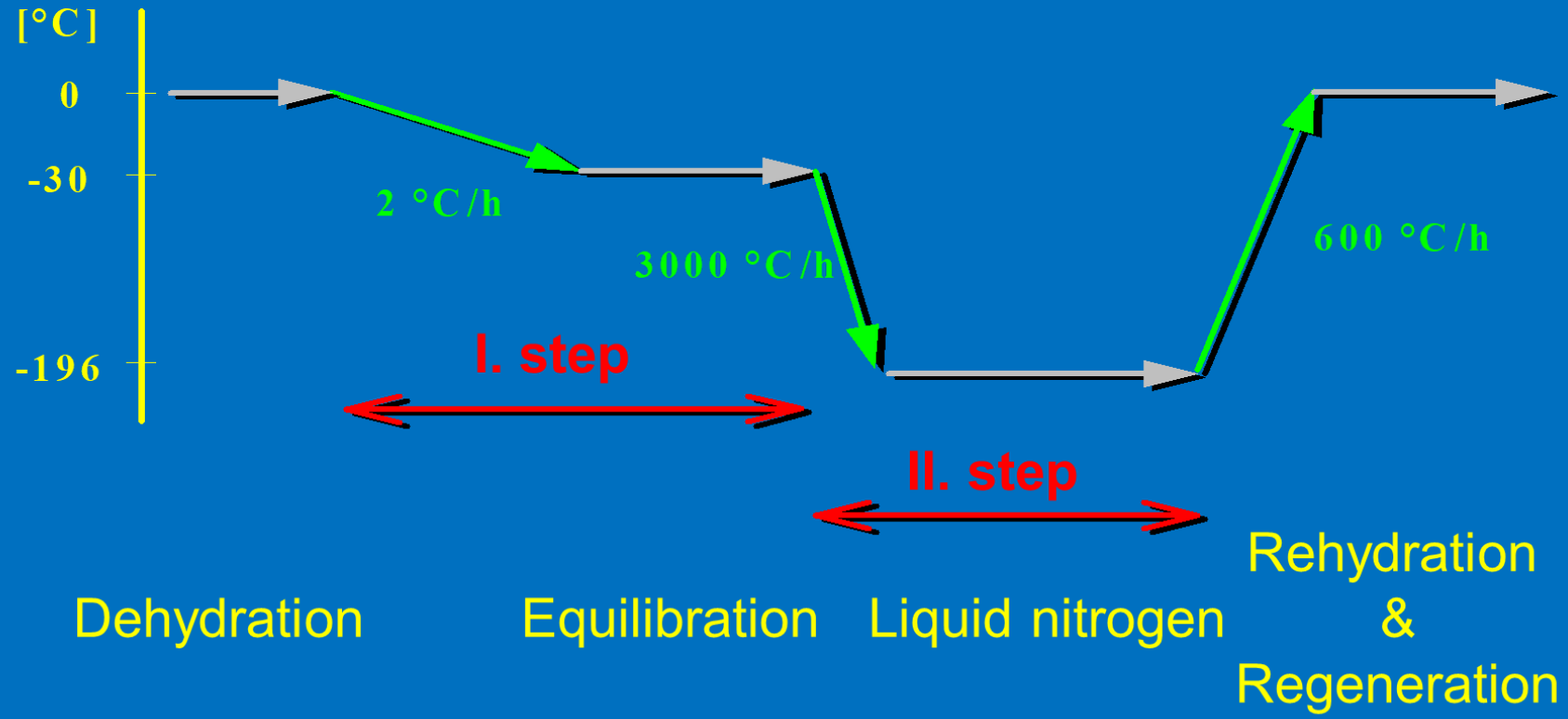
Two-step cryopreservation

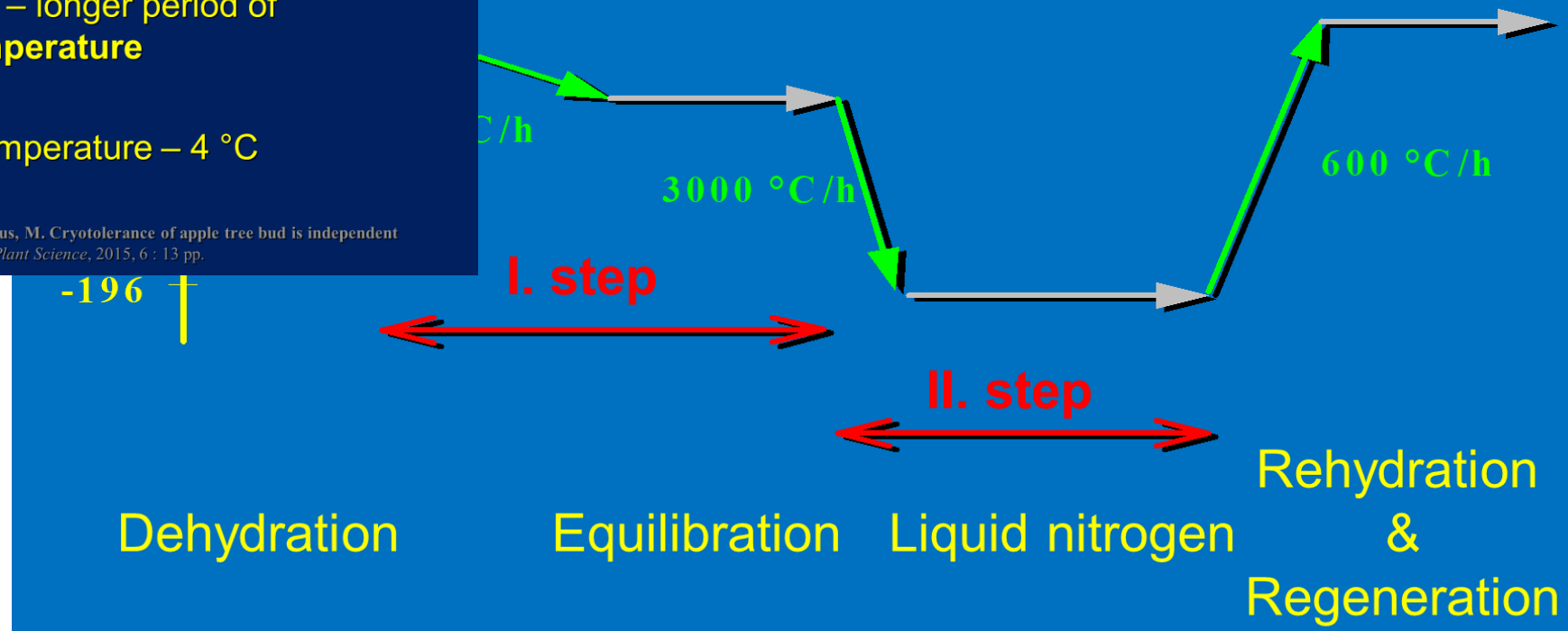


Sampling of buds

- Orchards - **ecodormant**
- Temperature – longer period of **subzero temperature**
- Keeping – temperature – 4 °C

Bilavčík, A., Zámečník, J., Faltus, M. Cryotolerance of apple tree bud is independent of endodormancy *Frontiers in Plant Science*, 2015, 6 : 13 pp.

o-step cryopreservation



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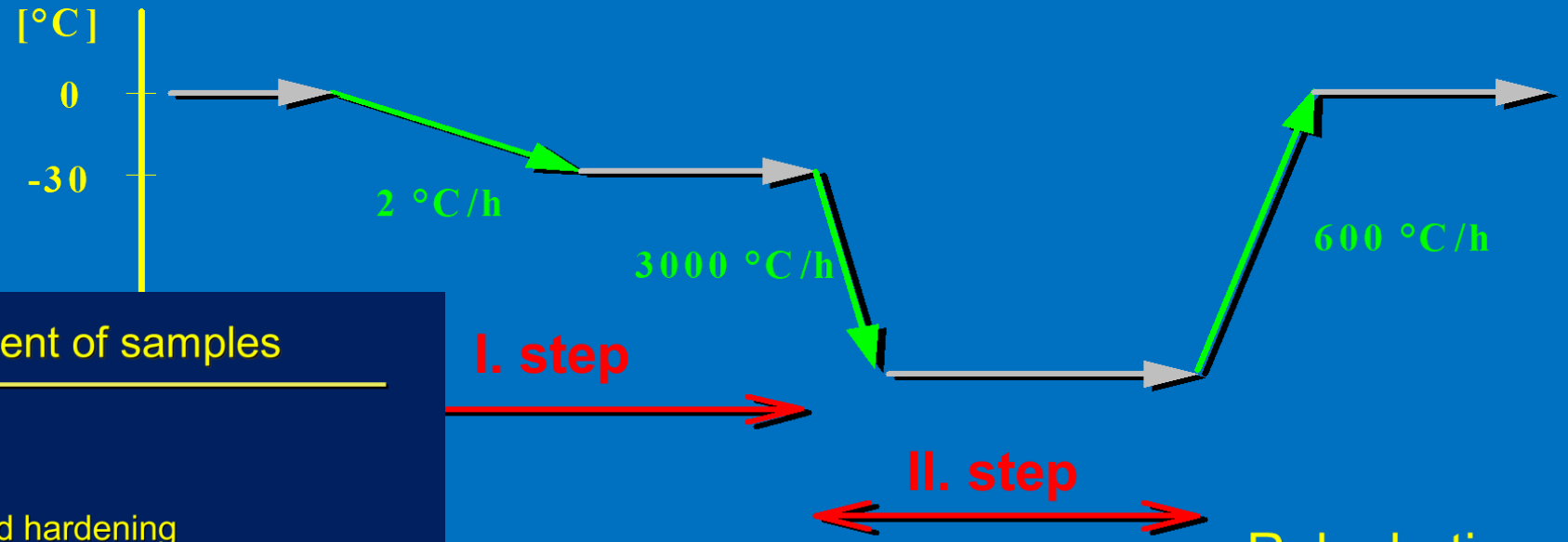


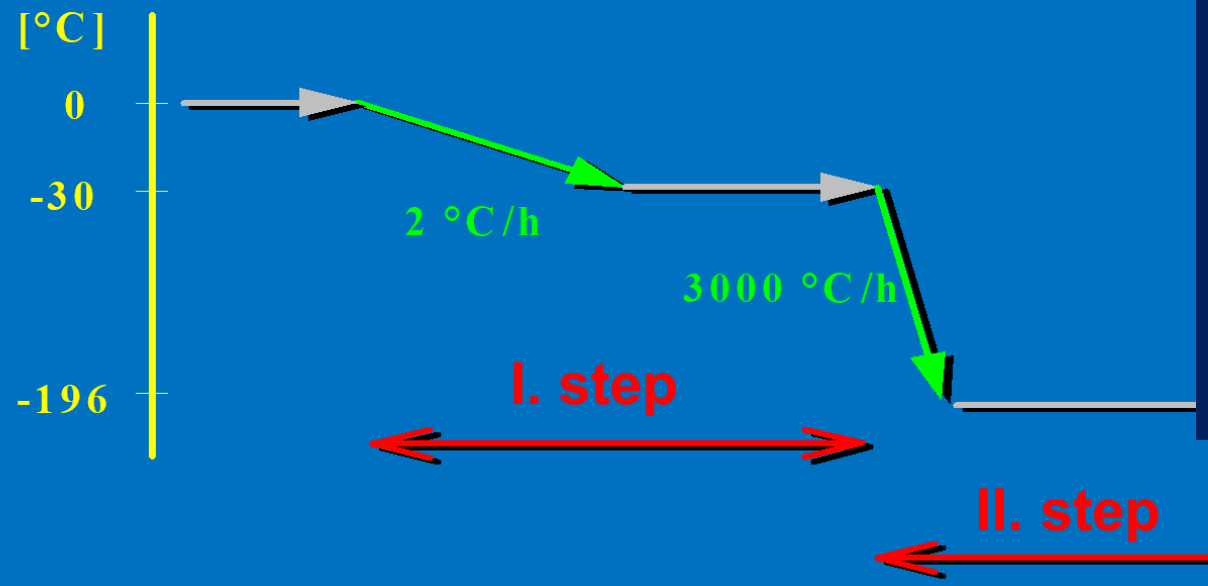
Pretreatment of samples

- Cutting
- Dehydration & cold hardening
- Thermal characteristics determination (DSC, DTA)

Zámečník, J., Faltus, M., Bilavčík, A., Kotková, R. Comparison of Cryopreservation Methods of Vegetatively Propagated Crops Based on Thermal Analysis In: Katkov, I. I. (ed.) Current Frontiers in Cryopreservation, InTech, Říjeka, Croatia, 2012, 333 - 357.

Zámečník, J, Bilavčík A., Faltus, M., Šesták, J. Water state in plants at low and ultra-low temperatures CryoLetters 24, 412-416

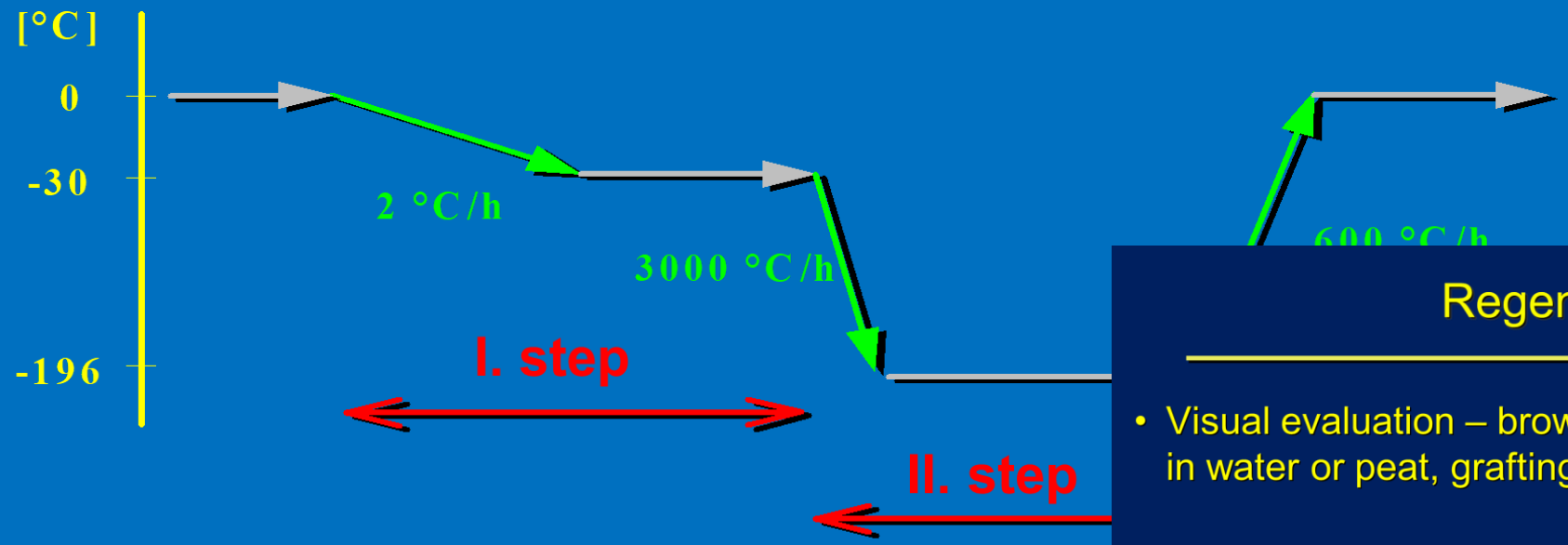
Two-step cryopreserva



Warming and rehydration

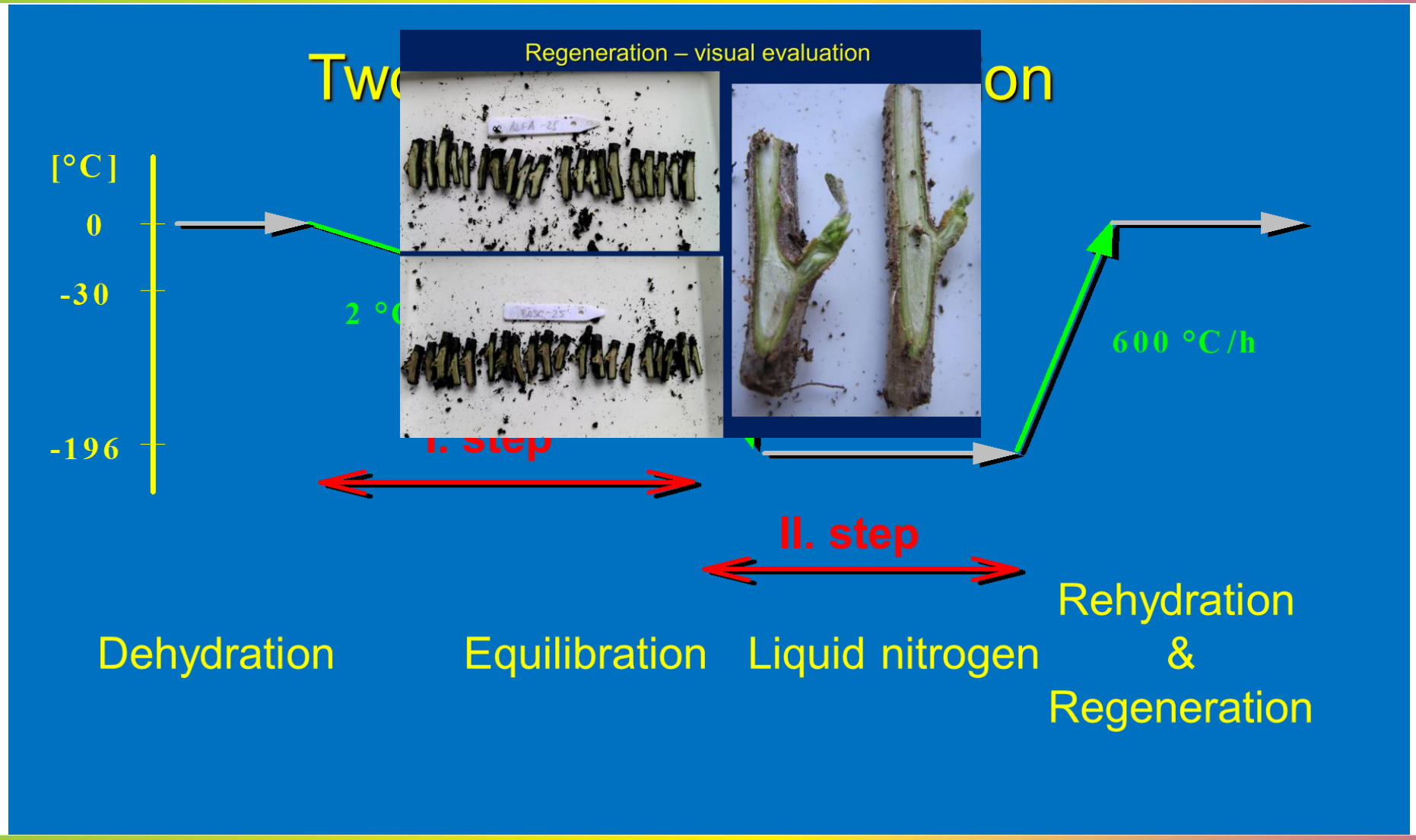
- Warming - at 4 °C
- Rehydration – moist white peat at 4 °C

Two-step cryopreservation

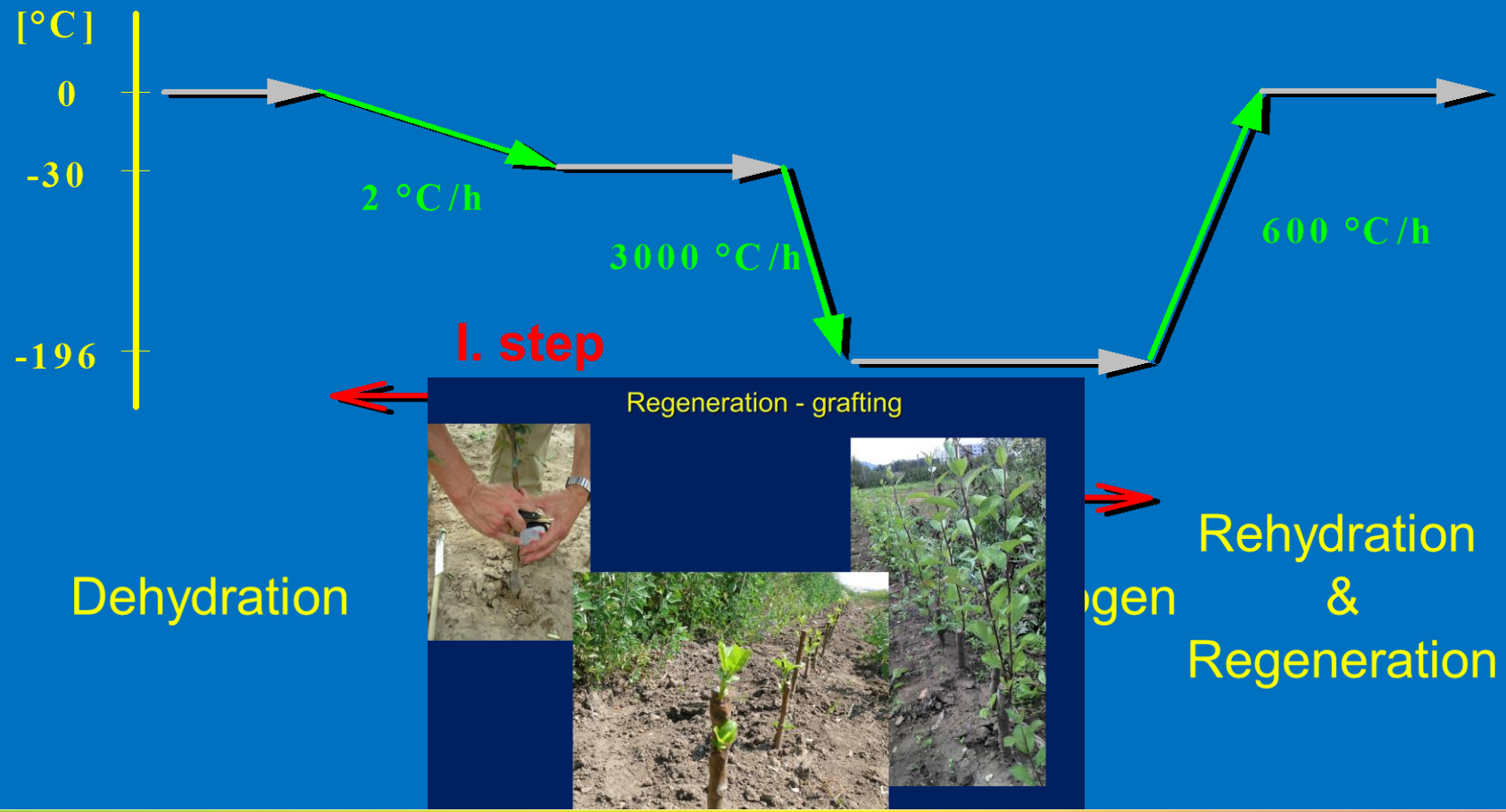


Regeneration

- Visual evaluation – browning of tissues, sprouting in water or peat, grafting
- Chip budding – orchard, glasshouse
- The end of May - beginning of July
- Evaluation – after 100 – 130 days


Two
on
Regeneration – visual evaluation
[°C]
0
-30
-196
2 °C
600 °C/h
I. step
II. step
Dehydration
Equilibration
Liquid nitrogen
Rehydration & Regeneration

Two-step cryopreservation



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Regeneration – visual evaluation

1. Step

Regeneration - grafting

Warming and rehydration

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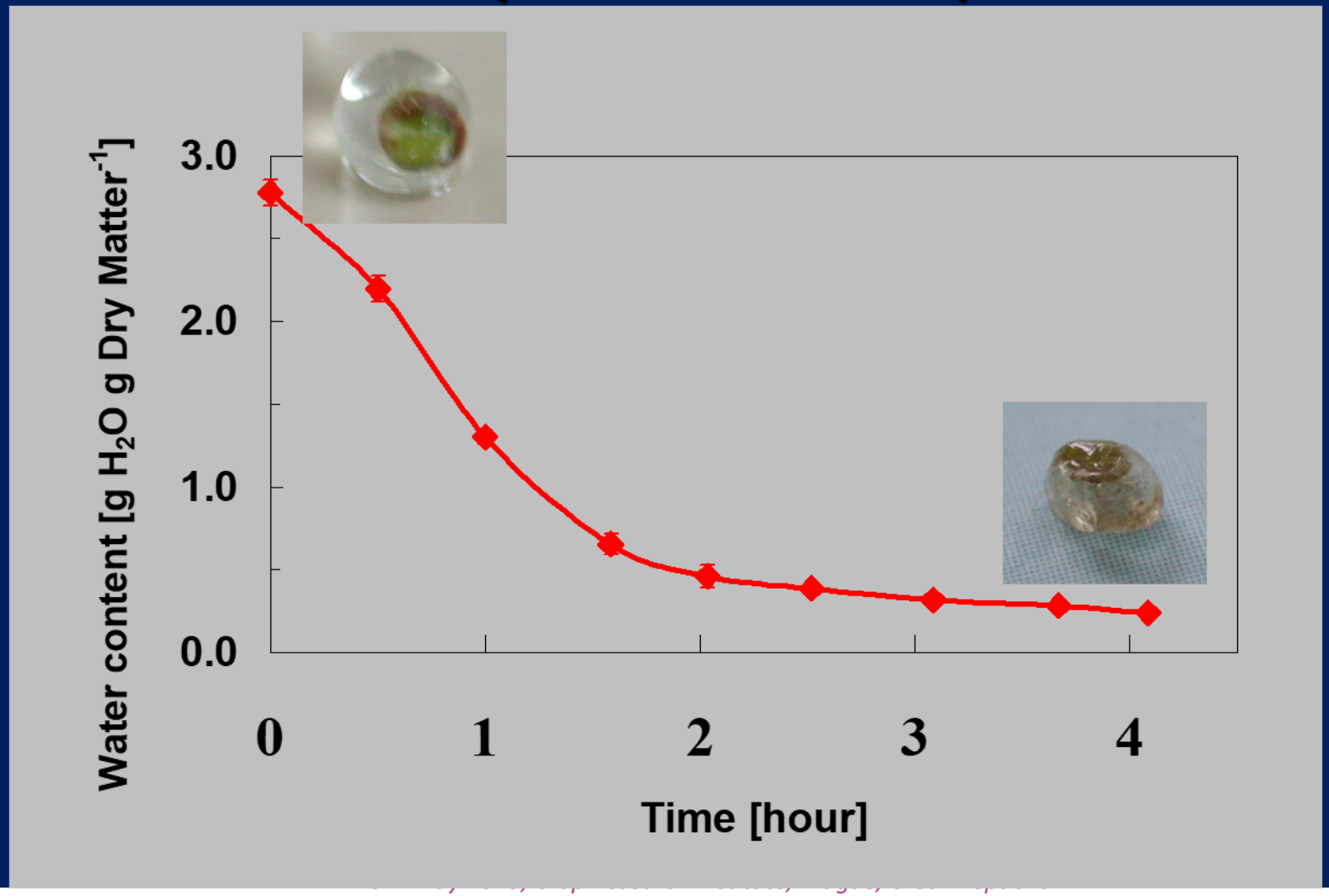
Dissection and encapsulation of *in vitro* cultures

Encapsulated shoot tip

Sedlák, J., Paprštein, F., **Bilavčík, A.**, **Zámečník, J.** Proliferation and cold hardening of in vitro grown apple shoot tips *Acta Horticulturae*, 2006, 725: 467 - 470

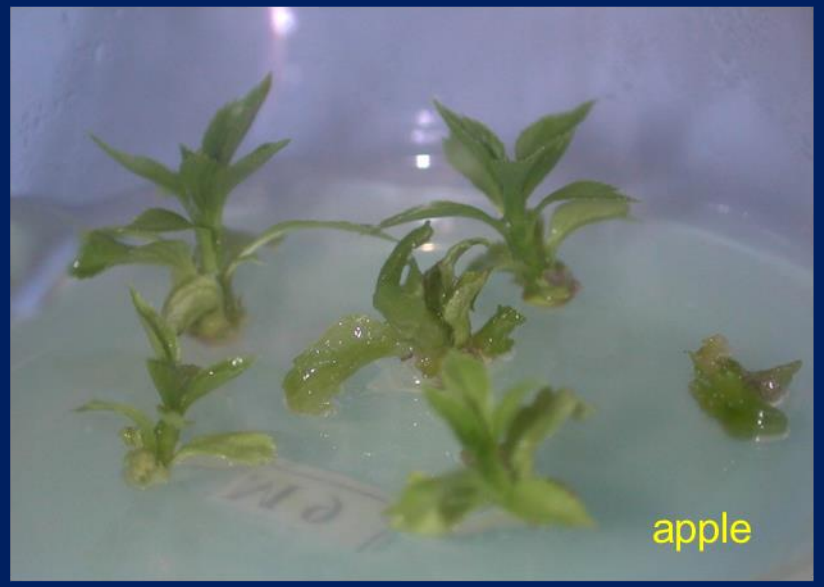
Dehydration

Encapsulated shoot tips



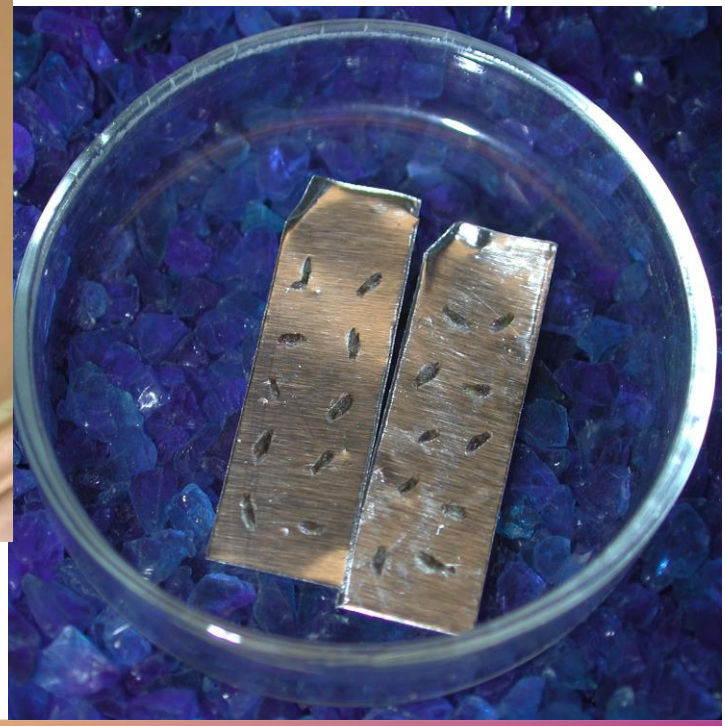
Regeneration of encapsulated shoot tips

Regenerating plants (30 days after thawing)



Regrowing shoot tip
(14 days after warming)

reservation protocols



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Cryopreservation protocols

Recovery - safe cryopreservation and recovery of samples

Cryopreservation protocols

Recovery - safe cryopreservation and recovery of samples

Minimal number of stored samples

- Number of stored samples – 120 shoot tips, 20 pcs for control recovery
- Minimal explant regeneration – 20 -30 %

Stefan Dussert probability tool

- Minimal number of stored shoot tips – 120 pcs
- Minimal size of control sample – 40 pcs
- Minimal explants recovery – 30%
- Minimal number of recoved shoot tips from total amount stored – 14 pcs

Cryotherapy

Virus elimination by cryopreservation

- Potato
- Hop
- Garlic
- Raspberry

Cryotherapy

POTATO

Method	Virus elimination		
	PLRV	PVY	PVS
thermotherapy	28%	24%	0%
chemotherapy	0%	22% *	**80%**
cryotherapy	**67%**	**64%**	0%

* Not succesfull for PVY- O

HOPS

Method	Virus elimination	
	ApMV	HMV
Thermotherapy	0%	0%
Chemotherapy	0%	0%
Cryotherapy	**15%**	**88%**

Thermal analysis as a tool for cryopreservation protocol development

Thermal Analysis – Differential Scanning Calorimetry

- **heat flow measurement** during temperature change – **assessment of heat capacity changes** – connected with changes of a **state of matter** – liquid vs solid, crystals vs glassy state

- the first-order transition events – **crystallization or melting** (connected with transition energy release)
  the second-order transition event – **glassy state**

- **freezable water content**

mal analysis as a tool for
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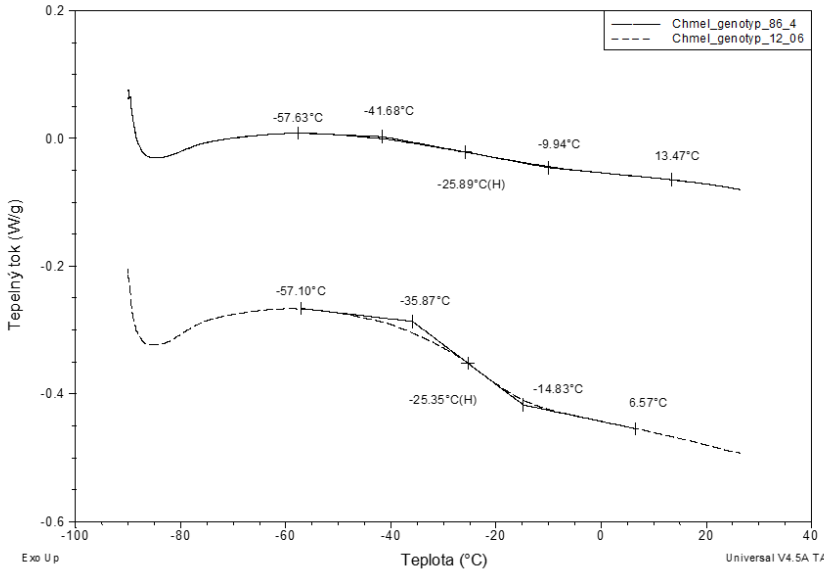

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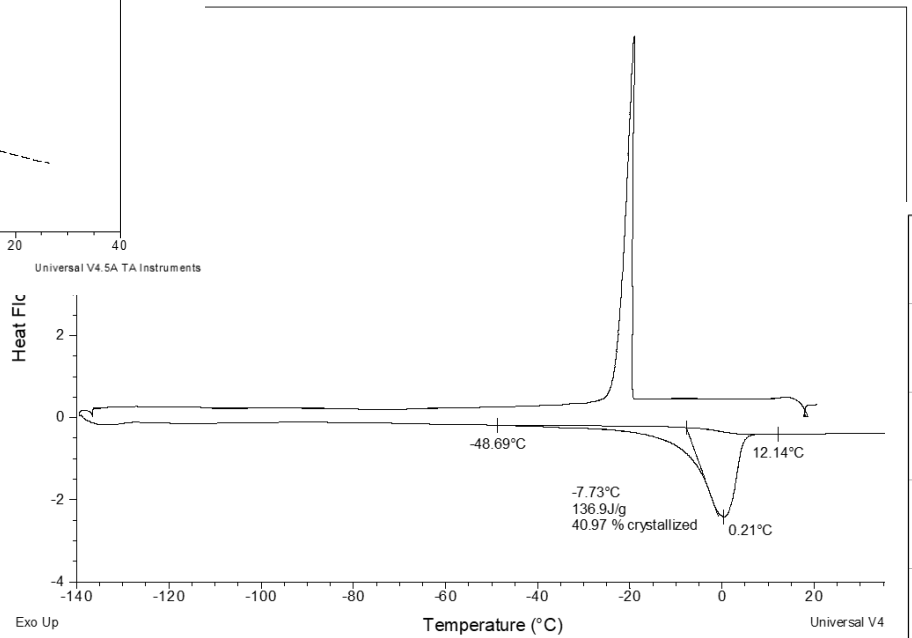
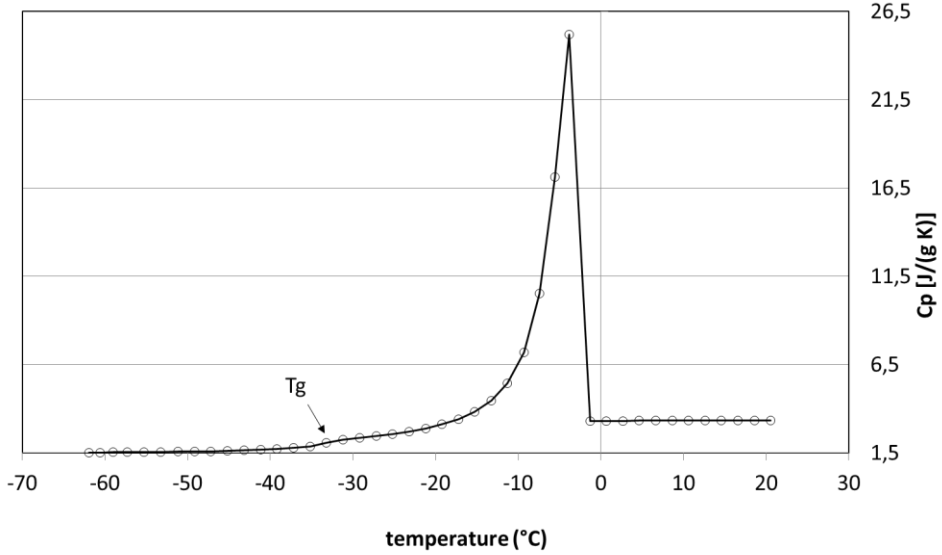


apacity changes – connected with



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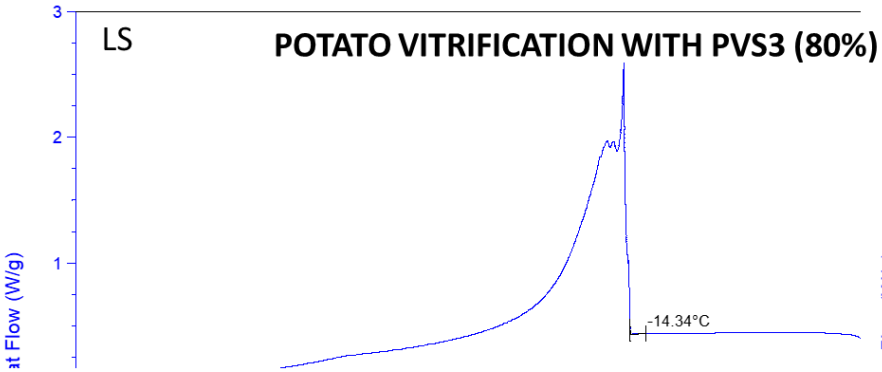
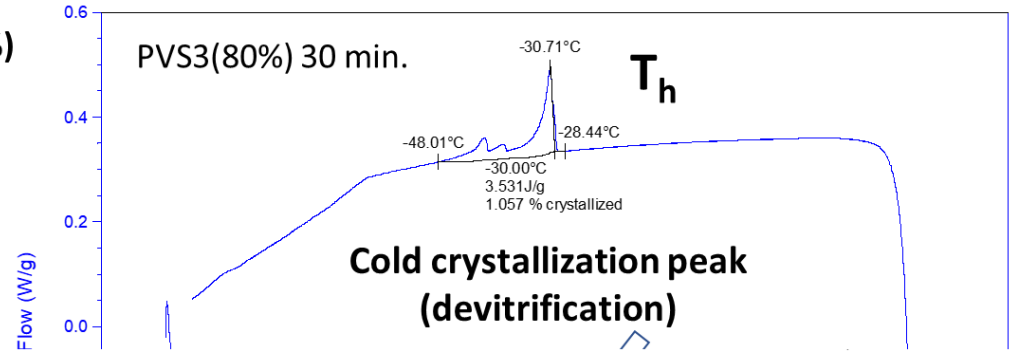
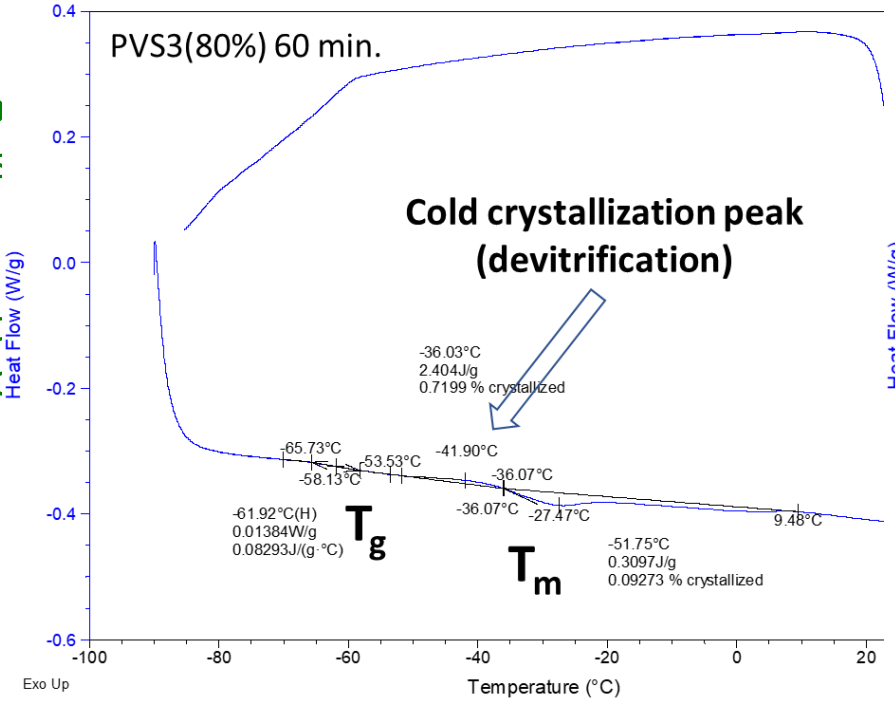
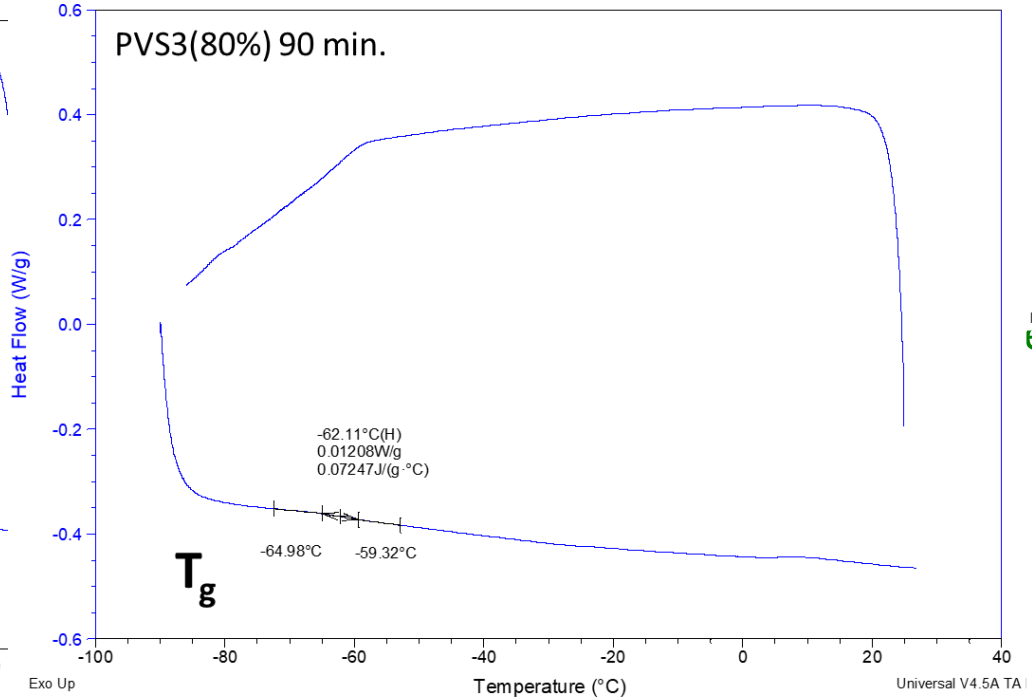
- **freezab**









Thermal analysis as a tool for cryopreservation protocol development

VITRIFICATION CONTROL BY DSC AT 10 °C/min.

CPA conc. group	SOLUTE CONCENTRATION (g / g)	CRYOPRESERVATION CONDITIONS	CRITICAL COOLING RATE CRITICAL WARMING RATE	FREEZING/MELTING during cooling / warming	GLASSY STATE	WATER CONTENT g (water) / g (dry mass)
1	0–0.5	Near-equilibrium freezing	CCR>10 °C/min.	T_h / T_m	$T_g' \approx T_{g(MFCP)}$	>1
2	0.5–0.6	Supercooling	CCR<10 °C/min. CWR>10 °C/min.	– / T_m	T_g	1–0.67
3	0.6	– devitrification sensitive	CWR≤10 °C/min.	– / – (T_m)	T_g	0.67
	0.7	Vitrification – optimal	CWR<10 °C/min.	– / –	T_g	0.4
	0.8	– „stable“	CWR~0 °C/min.	– / –	$T_g \approx T_{g(MFCP)}$	0.25
4	>0.8	Supersaturated solution	CCR>10 °C/min.	T_h / T_m	T_g''	<0.25

Prospects and limits of cryobanking

Goals:

- **Improving knowledge of cryotolerance**
- **Development of protocols for sensitive plant species and genotypes**
- **Complete the cryopreservation of selected types of crops of national importance**
- **Health status control of explants**
- **Sharing information about cryobanking**
- **Cooperation on international projects**

Limits:

- **unstable and insufficient funding of the cryobank**

Thank you for your attention!

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