Cryopreservation of temperate fruit species in Serbia

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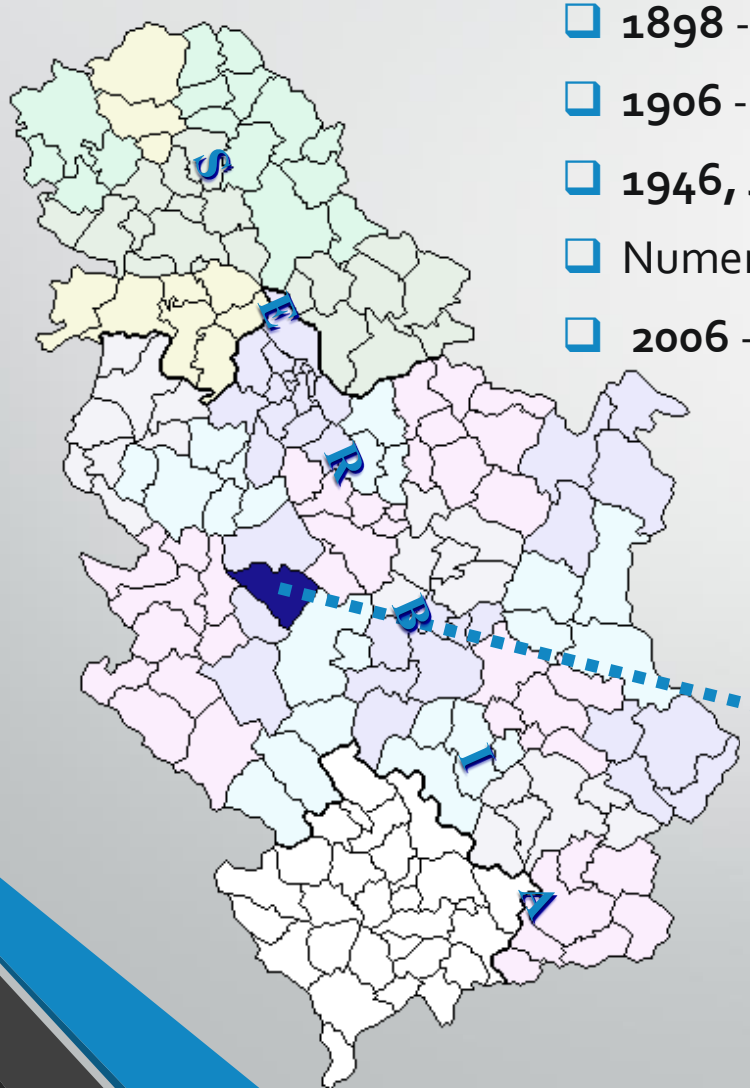
ИНСТИТУТ ЗА ВОЋАРСТВО ЧАЧАК
FRUIT RESEARCH INSTITUTE ČAČAK

Fruit Research Institute, Čačak

Research and Development Institute

- **1898** - Law on the improvement of fruit growing
- **1906** - fruit nursery
- **1946, July 17** – foundation day (Fruit Growing and Processing Station)
- Numerous transformations
- **2006** - Fruit Research Institute



Fruit Research Institute, Čačak

Departments

- Pomology and fruit breeding
- Fruit physiology
- Plant protection and certification of planting material
- Technology of fruit growing
- Fruit processing technology
- Experimental fields

Research & development laboratories

- Chemical analysis
- Phytopathology
- Tissue culture *in vitro*

Collection, characterisation and evaluation

of autochthonous genotypes of pome, stone, kernel and small fruit species

- Besides the cultivated fruit species and cultivars, a large number of wild and related species have been registered in Serbia.
- *Ex situ* collection of Fruit Research Institute comprises more than **800** genotypes of different fruit species.
- Breeders are currently involved in characterization and evaluation of considerable number of landraces, especially those that exhibit favorable traits (resistance to pests and diseases, long shelf life, etc.).
- The significant outcome of the Cryoplum project are *in vivo* and *in vitro* collections of nine autochthonous plum cultivars that are maintained in the greenhouse and laboratories of the Fruit Research Institute and are available for various purposes (conservation, propagation, breeding, exchange, morphological characterization and agronomic evaluation).

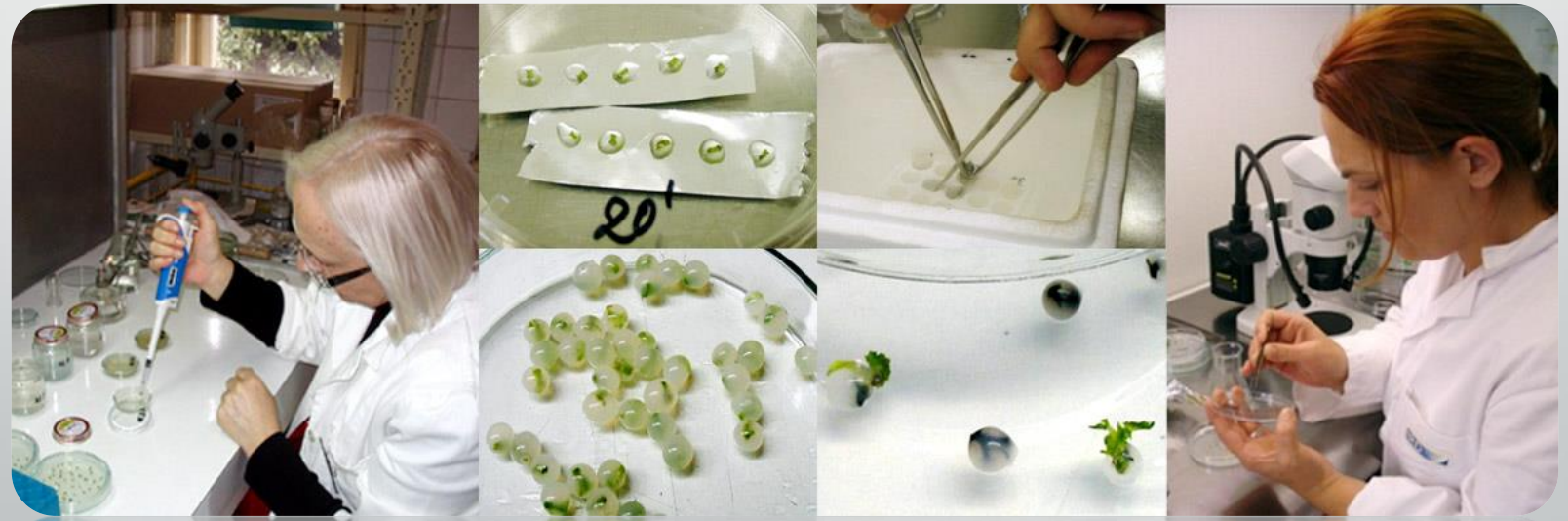
Our interest?

To reestablish fruit gene bank of Serbia employing, among others, different *in vitro* cryopreservation techniques.

Department of Fruit Physiology

Cryopreservation *in vitro*

- Encapsulation dehydration
  - Vitrification
- Droplet vitrification
  - V cryo-plate
  - D cryo-plate



Projects

COST 863 STMS

Participant: Đurđina Ružić

Title: The application and the elaboration of the protocol for encapsulation-dehydration, vitrification and droplet vitrification techniques in raspberry (*Rubus idaeus* L.)

Place: CRA - Fruit Tree Research Centre of Rome, Italy

Supervisor: Dr Carmine Damiano and Dr Emilia Caboni

COST 871 STMS

Participant: Tatjana Vujović

Title: Cryopreservation of autochthonous plum genotypes (*P. insititia* L. and *P. cerasifera* Ehrh.) using encapsulation-dehydration and droplet vitrification techniques

Place: IRD, Montpellier, France

Supervisor: Dr Florent Engelmann

SERBIAN-FRENCH BILATERAL SCIENTIFIC COOPERATION (Program 'Pavle Savic' – 'Partnership Hubert Curien')

Project title: Application of droplet-vitrification and V cryo-plate methods in cryopreservation of *Prunus* genetic resources

Institutions: IRD and INRA, Montpellier, France and FRI, Čačak, Serbia

Duration: 2014–2015

Projects

BILATERAL SCIENTIFIC COOPERATION BETWEEN SERBIA AND CROATIA

Project title: In vitro propagation, conservation and quantification of biological activity of fruits of small fruit species and grapevine
Institutions: University of Zagreb – Faculty of Agriculture, Croatia and FRI, Čačak, Serbia
Duration: 2019–2022

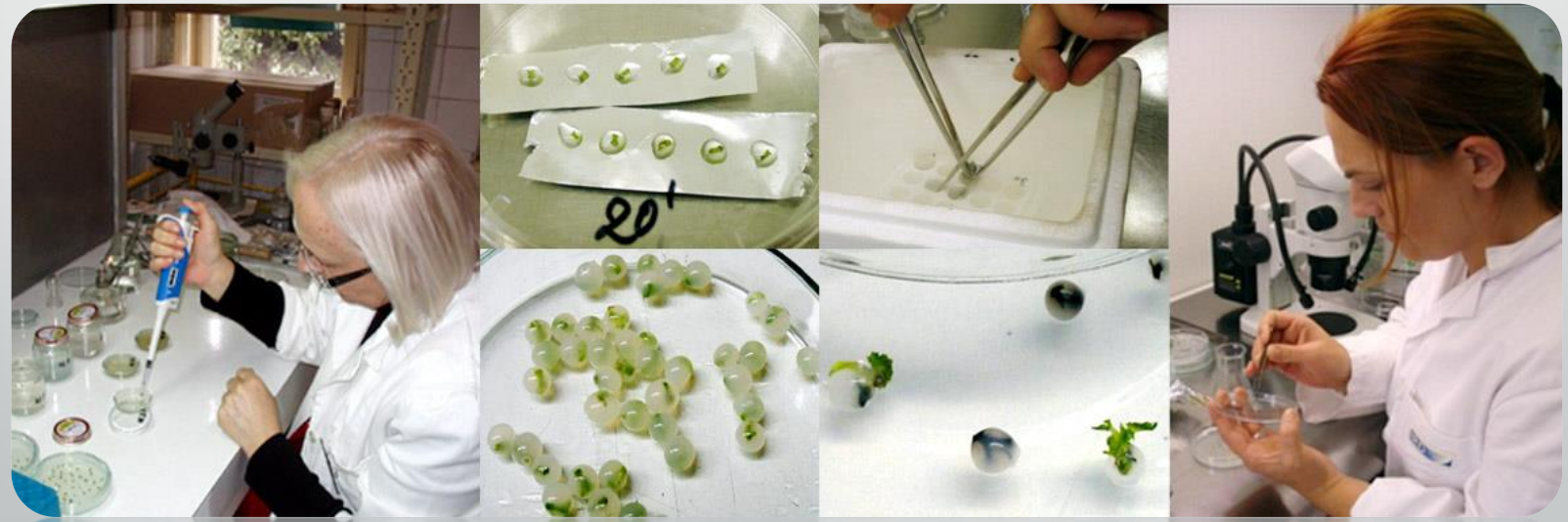
SCIENCE FUND OF THE REPUBLIC OF SERBIA
Program for excellent projects of young researchers (PROMIS)

Project title: Conservation and plum pox virus eradication from Serbian autochthonous plum genotypes using cryotechniques - CryoPlum
Institutions: FRI, Čačak, Serbia
Duration: 2020–2022

COST ACTION CA21157

Project title: European Network for Innovative Woody Plant Cloning (COPYTREE)
Duration: 2022–2026

What we have done so far



Vitrification

1. Ružić Dj., Vujović T., Cerović R. (2014): Cryopreservation of cherry rootstock Gisela 5 using vitrification procedure. Horticultural Science (Prague), 41, 2: 55–63.

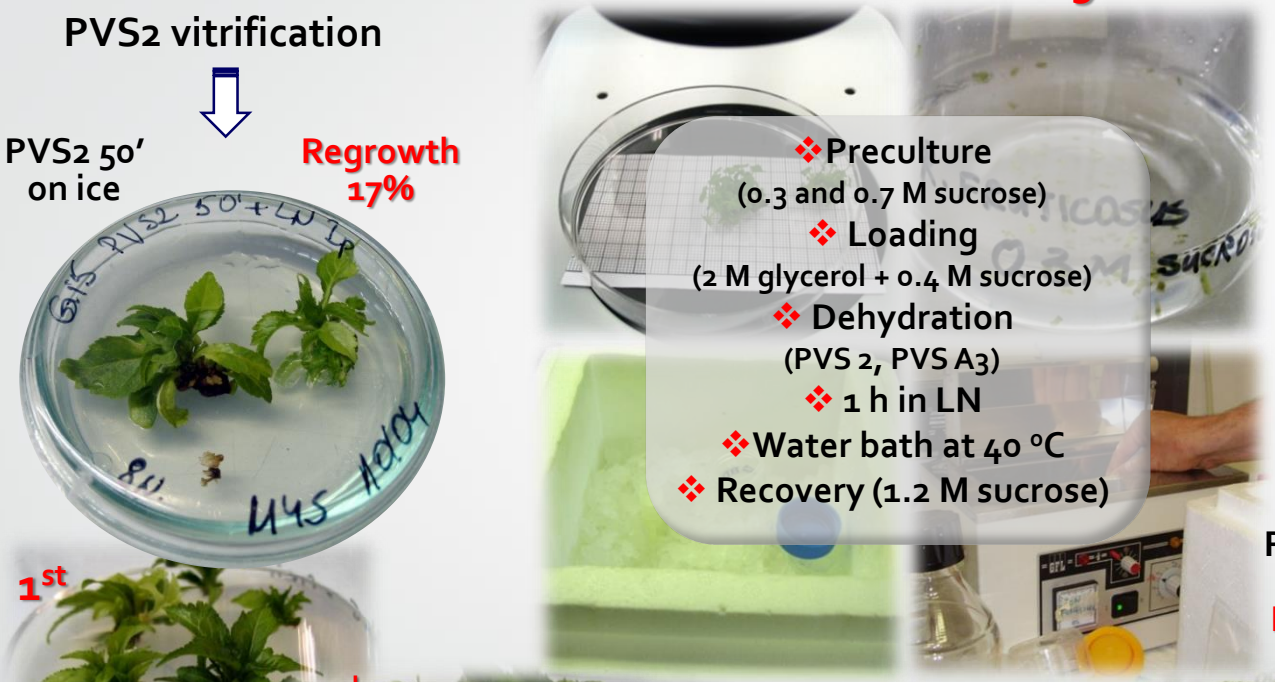
Cherry plum, autochthonous plums
Sitnica
Crvena Ranka

PVS2 vitrification

No regrowth

Rootstock 'Gisela 5'

- Preculture (0.3 and 0.7 M sucrose)
- Loading (2 M glycerol + 0.4 M sucrose)
- Dehydration (PVS 2, PVS A3)
- 1 h in LN
- Water bath at 40 °C
- Recovery (1.2 M sucrose)



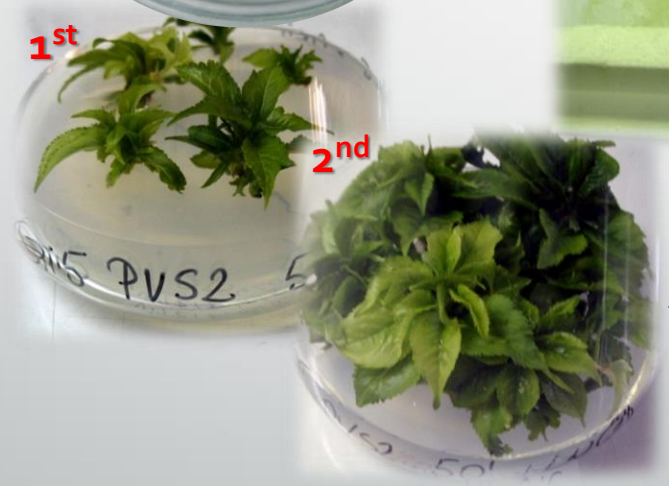
PVS2 vitrification

PVS2 50' on ice

Regrowth 17%

1st

2nd

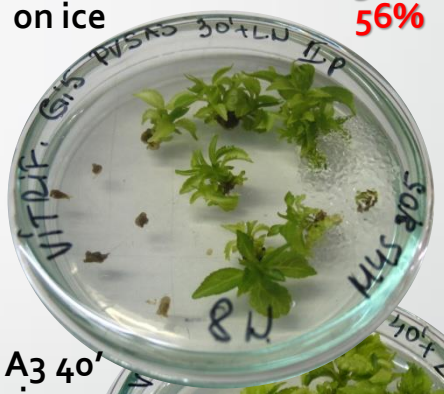


Multiplication in successive subcultures after regrowth

PVS A3 vitrification

PVS A3 30' on ice

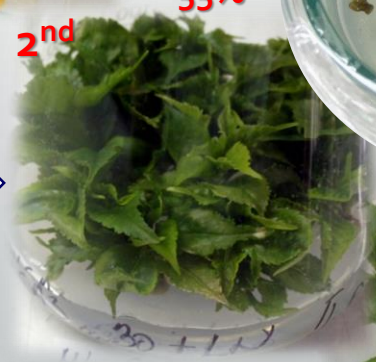
Regrowth 56%



PVS A3 40' on ice

Regrowth 55%

2nd



1st

Vitrification

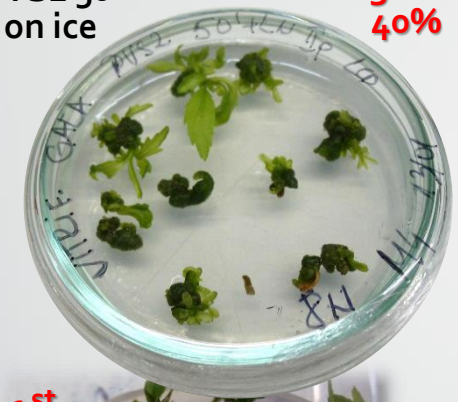
1. Vujović T., Ružić Đ., Cerović R. (2021): Cryopreservation of apple shoot tips by vitrification and subsequent plant regeneration. Acta Horticulturae, 1308: 33–40.

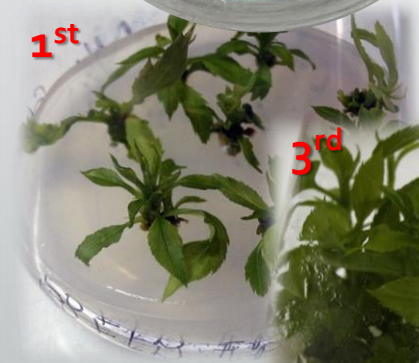
Apple Gala Must

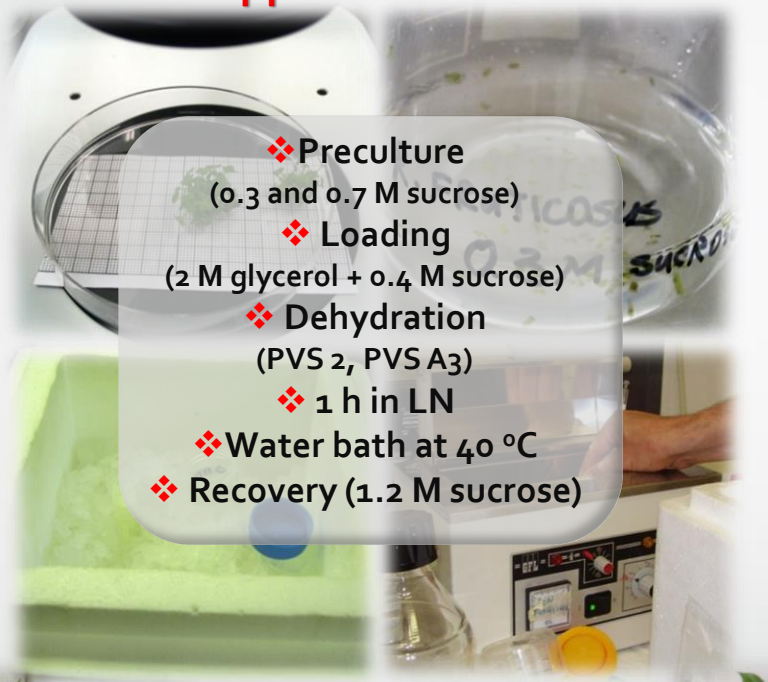
PVS2 vitrification

PVS2 50' on ice

Regrowth 40%





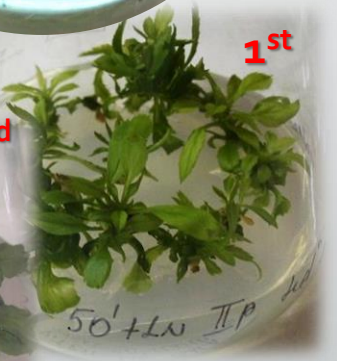


- Preculture (0.3 and 0.7 M sucrose)
- Loading (2 M glycerol + 0.4 M sucrose)
- Dehydration (PVS 2, PVS A3)
- 1 h in LN
- Water bath at 40 °C
- Recovery (1.2 M sucrose)

PVS A3 vitrification

PVS A3 50' on ice

Regrowth 75%



Multiplication and rooting in successive subcultures after regrowth

Droplet vitrification

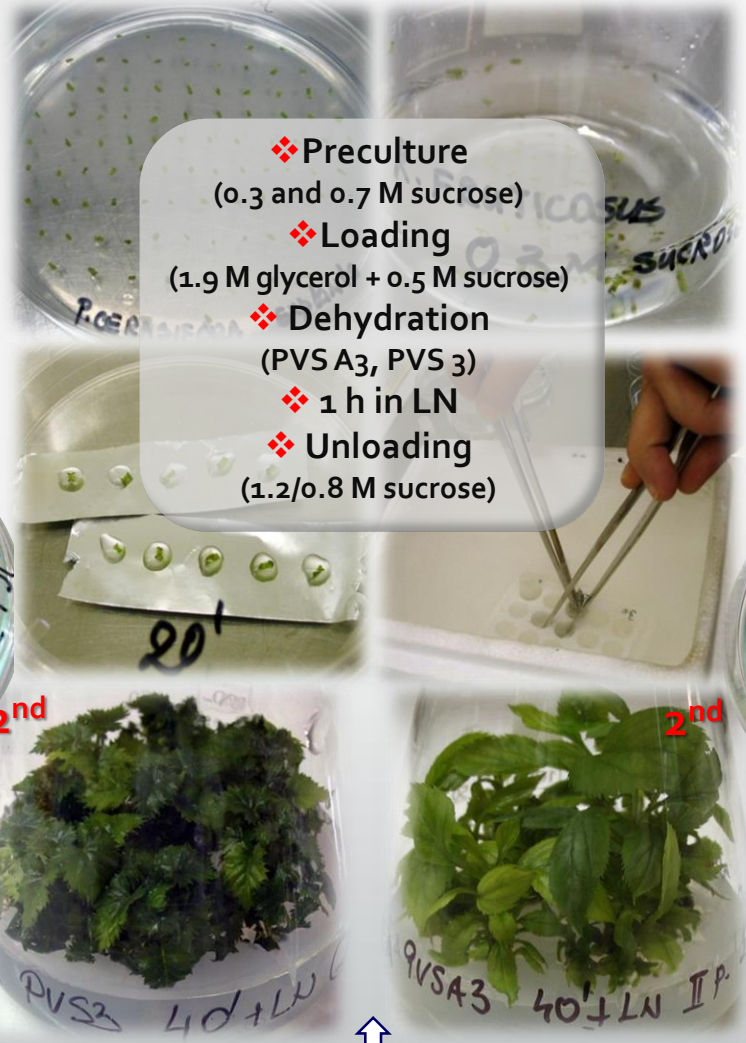
1. Vujović T., Sylvestre I., Ružić Đ., Engelmann F. (2011): Droplet-vitrification of apical shoot tips of *Rubus fruticosus* L. and *Prunus cerasifera* Ehrh. Scientia Horticulturae, 130: 222–228; 2. Vujović T., Ružić Đ., Cerović R. (2015): Optimization of droplet vitrification protocol for cryopreservation of *in vitro* grown blackberry shoot tip. Acta Horticulturae, 1099: 595–601; 3. Vujović T., Ružić Đ., Cerović R. (2017): Effect of the duration of liquid nitrogen storage on the regrowth of blackberry cryopreserved by droplet vitrification. Contemporary Agriculture, 66, 1–2: 44 - 50.

Blackberry 'Čačanska Bestrna'

PVS A3 40' on ice → Regrowth 62%

PVS A3 30' on ice → Regrowth 55%

PVS 3 40' room t° → Regrowth 90%



Apple 'Gala Must'

Regrowth 70% ← PVS A3 40' on ice

PVS A3 30' on ice → Regrowth 65%

PVS 3 50' room t° ← Regrowth 50%

Multiplication in successive subcultures after regrowth

4. Vujović T., Ružić Đ., Vranić D., Marjanović T. (2020): Cryopreservation in vitro of apple shoot tips following droplet-vitrification. Acta Horticulturae, 1289: 1–8.

Droplet vitrification

1. Ružić Dj., Vujović T., Cerović R. (2013): Cryopreservation of cherry rootstock Gisela 5 (*Prunus cerasus* × *Prunus canescens*) shoot tips by droplet-vitrification technique. Journal of Horticultural Research, 21, 2:79–85.

Rootstock 'Gisela 5'

PVS A3 vitrification

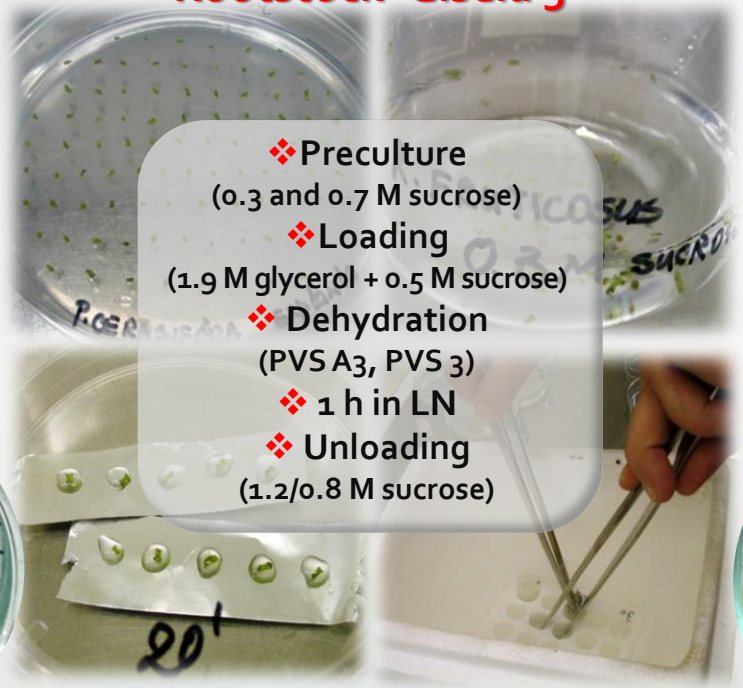
Regrowth 40%

PVS A3 40' on ice

PVS A3 40' rt°

Regrowth 38%

- Preculture (0.3 and 0.7 M sucrose)
- Loading (1.9 M glycerol + 0.5 M sucrose)
- Dehydration (PVS A3, PVS 3)
- 1 h in LN
- Unloading (1.2/0.8 M sucrose)



Multiplication ⇦ in ⇨ successive subcultures after regrowth

PVS 3 vitrification

Regrowth 38%

PVS 3 90' rt°

PVS 3 60' rt°

Regrowth 40%

Droplet vitrification

1. Vujović T., Sylvestre I., Ružić Dj., Engelmann F. (2012): Cryopreservation of cherry plum and blackberry shoot tips by droplet-vitrification. Proceedings of Final Meeting of COST Action 871 – Cryopreservation of Crop Species in Europe, Angers (France), pp. 163–166.
2. Vujović T., Ružić Dj., Cerović R. (2015): Cryopreservation in vitro of autochthonous *Prunus* sp. by droplet-vitrification. Biologia, 70, 10: 1359–1365.

Cherry plum

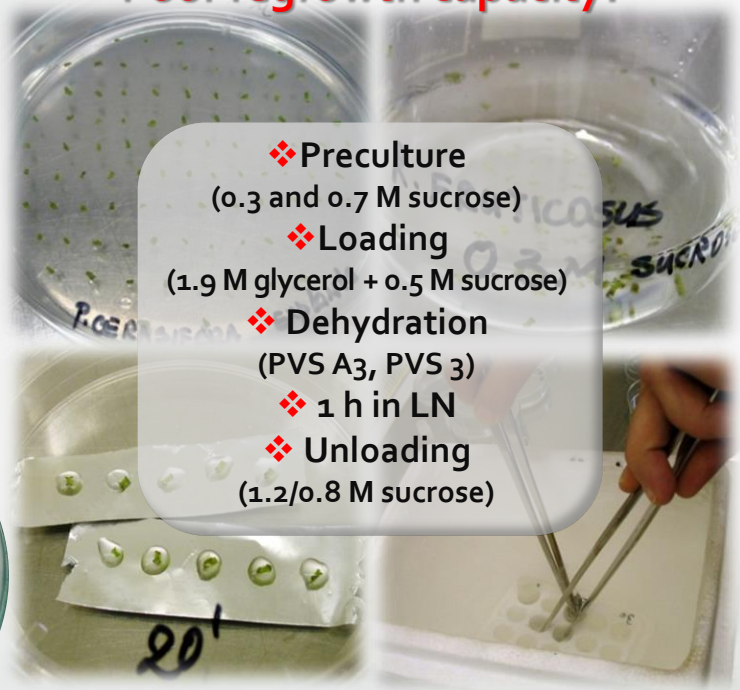
Regrowth 28%

PVS A3 30' rt° 60' unl.

PVS A3 40' rt° 60' unl. Regrowth 27%

Poor regrowth capacity!

- Preculture (0.3 and 0.7 M sucrose)
- Loading (1.9 M glycerol + 0.5 M sucrose)
- Dehydration (PVS A3, PVS 3)
- 1 h in LN
- Unloading (1.2/0.8 M sucrose)



Plum 'Crvena Ranka'

Regrowth 36%

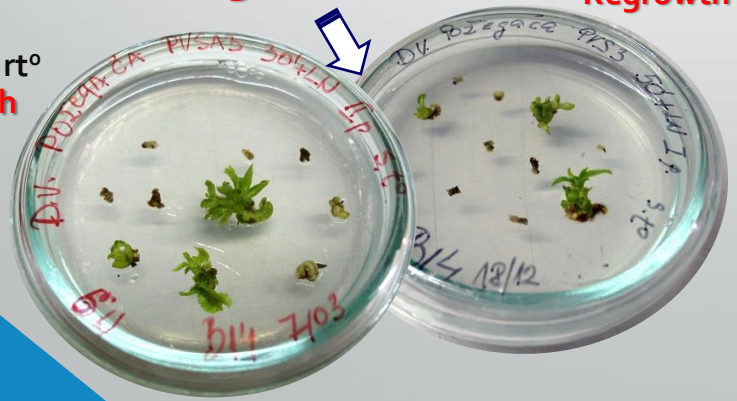
PVS A3 30' rt°

PVS A3 40' rt°/60' unl. Regrowth 34%

Plum 'Požegača'

PVS A3 30' rt° Regrowth 15%

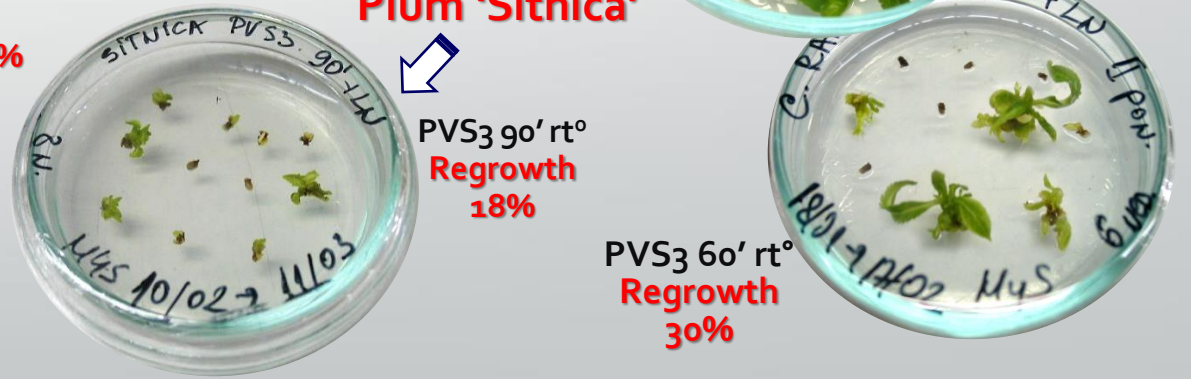
PVS 3 50' rt° Regrowth 10%



Plum 'Sitnica'

PVS3 90' rt° Regrowth 18%

PVS3 60' rt° Regrowth 30%



V cryo-plate

1. Vujović T., Chatelet Ph., Ružić Đ., Engelmann F. (2017): Cherry plum & plum (Prunus sp.), V cryo-plate; D cryo-plate. In: 'Manual of cryopreservation methods using cryo-plate. V and D cryo-plate procedures as an effective protocol for cryobanks', Nino T., Matsumoto T., Yamamoto S-I., Maki S., Tanaka D, Engelmann F. (eds.), pp. 66–69.

INTER-LABORATORY VALIDATION

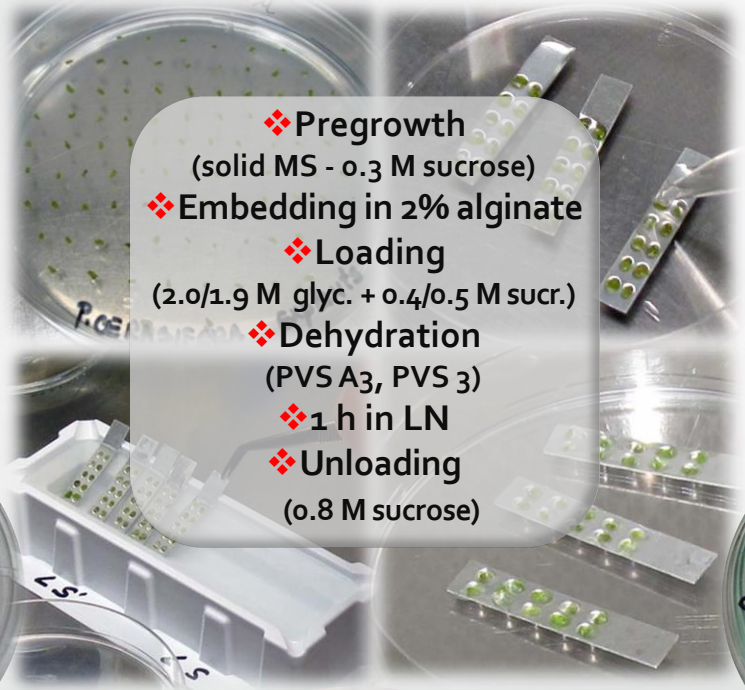
Cherry plum

Regrowth 42%

C4-PVS A3 30' rt°

LS1-PVS A3 30' rt°

Regrowth 56%



- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0/1.9 M glyc. + 0.4/0.5 M sucr.)
- Dehydration (PVS A3, PVS 3)
- 1 h in LN
- Unloading (0.8 M sucrose)

Plum 'Požegača'

Regrowth 34%

C4-PVS A3 30' rt°

LS1-PVS A3 30' rt°

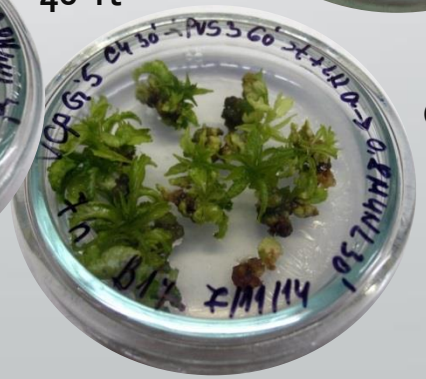
Regrowth 45%

Regrowth 96%

C4-PVS A3 40' rt°

LS1-PVS A3 40' on ice

Regrowth 26%



C4-PVS3 60' rt°

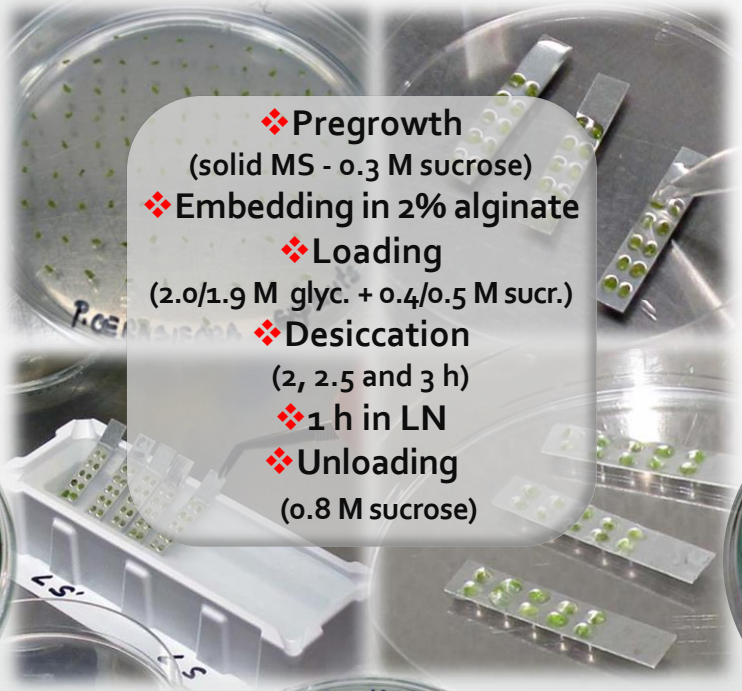
Regrowth 85%

Rootstock Gisela 5

D cryo-plate

2. Vujović T., Chatelet Ph., Ružić Đ., Engelmann F. (2015): Cryopreservation of Prunus sp. using aluminium cryo-plates. Scientia Horticulturae, 195: 173–182.
3. Vujović T., Ružić Đ., Marjanović T., Jevremović D. (2020): Application of V and D cryo-plate methods for the cryopreservation of cherry rootstock Gisela 5. Book of Proceedings of the XI International Scientific Agricultural Symposium 'Agrosym 2020', pp. 62–68.

INTER-LABORATORY VALIDATION



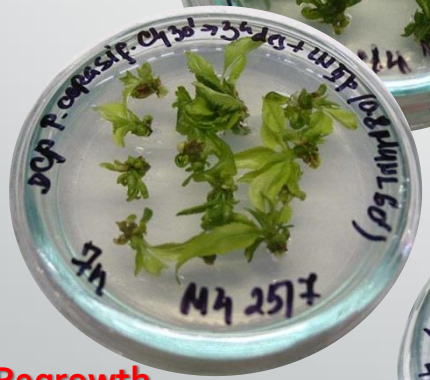
- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0/1.9 M glyc. + 0.4/0.5 M sucr.)
- Desiccation (2, 2.5 and 3 h)
- 1 h in LN
- Unloading (0.8 M sucrose)

Cherry plum

Regrowth 58% — 2 h desicc.

Regrowth 65% — 2.5 h desicc.

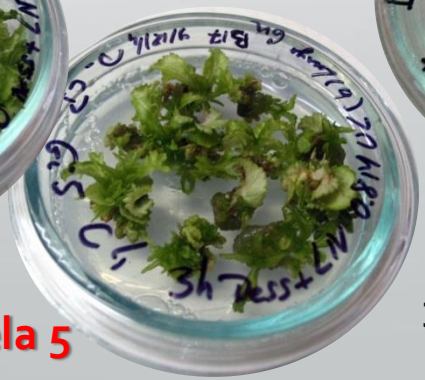
Regrowth 78% — 3 h desicc.



Regrowth 46% — 2 h desicc.

Regrowth 67% — 2.5 h desicc.

Rootstock Gisela 5

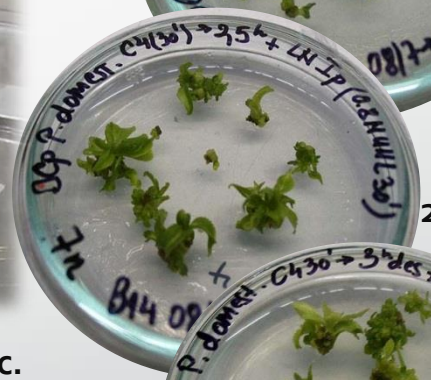


Regrowth 67% — 3 h desicc.

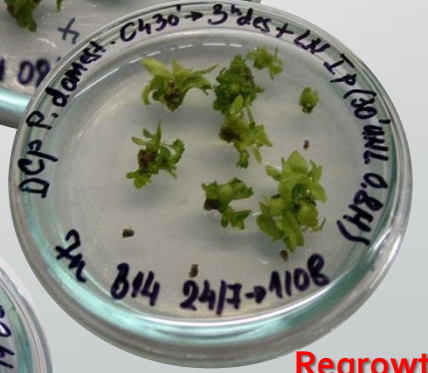
Plum 'Požegača'

Regrowth 48% — 2 h desicc.

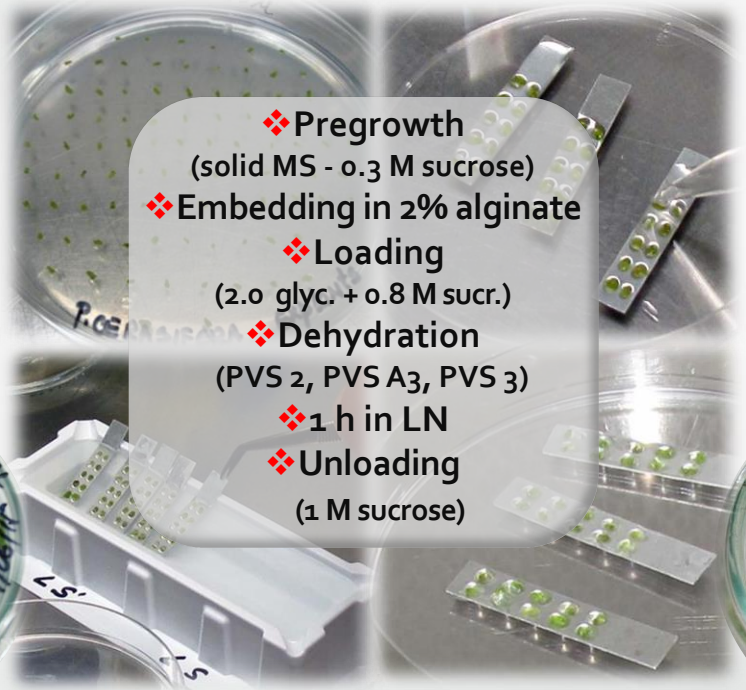
Regrowth 47% — 2.5 h desicc.



Regrowth 30% — 3 h desicc.



V cryo-plate



- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0 glyc. + 0.8 M sucr.)
- Dehydration (PVS 2, PVS A3, PVS 3)
- 1 h in LN
- Unloading (1 M sucrose)

Strawberry 'Clery'

Regrowth 50%

LS2-PVS2 50' rt°

LS2-PVS A3 50' rt° Regrowth 46%

LS2-PVS3 50' rt° Regrowth 63%

LS2-PVS A3 40' rt° Regrowth 36%

Amelancier alnifolia

LS2-PVS3 40' rt° Regrowth 50%

Raspberry 'Willamtte'

LS2-PVS3 50' rt° Regrowth 20%

Blueberry 'Toro'

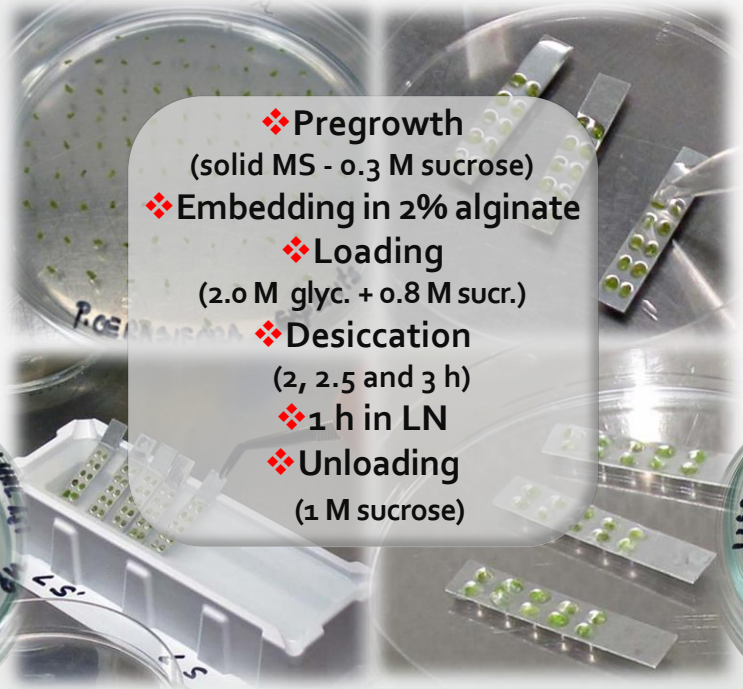
Regrowth 69%

LS2-PVS 2 50' rt°

LS2-PVS A3 50' rt° Regrowth 80%

LS2-PVS3 50' rt° Regrowth 68%

D cryo-plate



- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0 M glyc. + 0.8 M sucr.)
- Desiccation (2, 2.5 and 3 h)
- 1 h in LN
- Unloading (1 M sucrose)

Strawberry 'Clery'

Regrowth 63% — LS2 2 h desicc.

Regrowth 83% — LS2, 2.5 h desicc.

Regrowth 46% — LS2 , 3 h desicc.

Raspberry 'Willamtte'

No regrowth

Blueberry 'Toro'

Regrowth 78% — LS2, 2 h desicc.

Regrowth 77.3% — LS2, 2.5 h desicc.

Regrowth 83% — LS2, 3 h desicc.

Amelancier alnifolia

Regrowth 20.% — LS2, 2 h desicc.

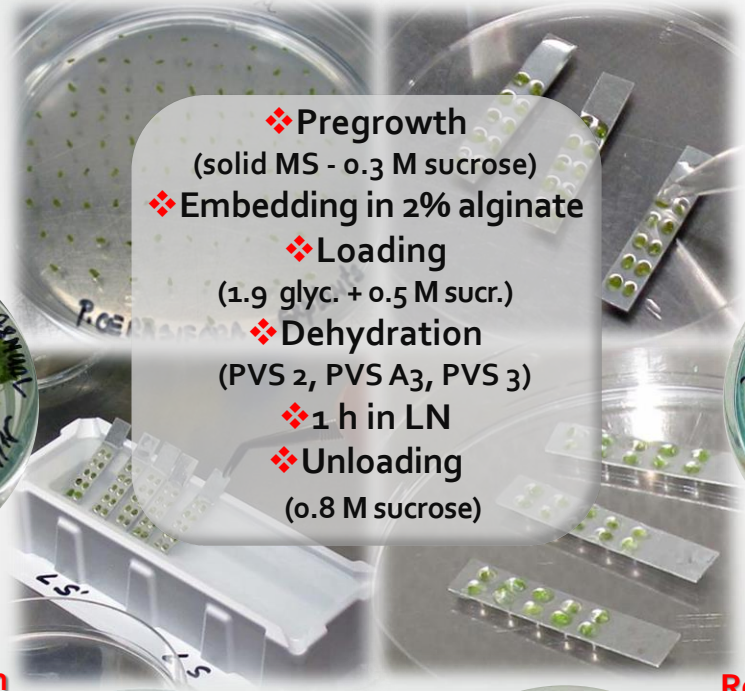
Regrowth 13% — LS2, 3 h desicc.

V cryo-plate

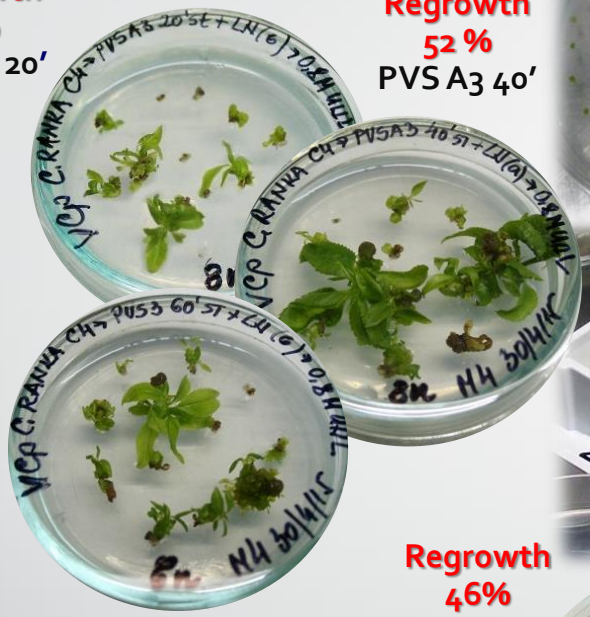
1. Vujović T., Jevremović D., Marjanović T., Ružić Đ. (2021): Cryopreservation of Serbian autochthonous plum 'Crvena Ranka' using aluminium cryo-plates. Genetika, 53, 1: 283–294.

CryoPlum

- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (1.9 glyc. + 0.5 M sucr.)
- Dehydration (PVS 2, PVS A3, PVS 3)
- 1 h in LN
- Unloading (0.8 M sucrose)



Plum 'Crvena Ranka'

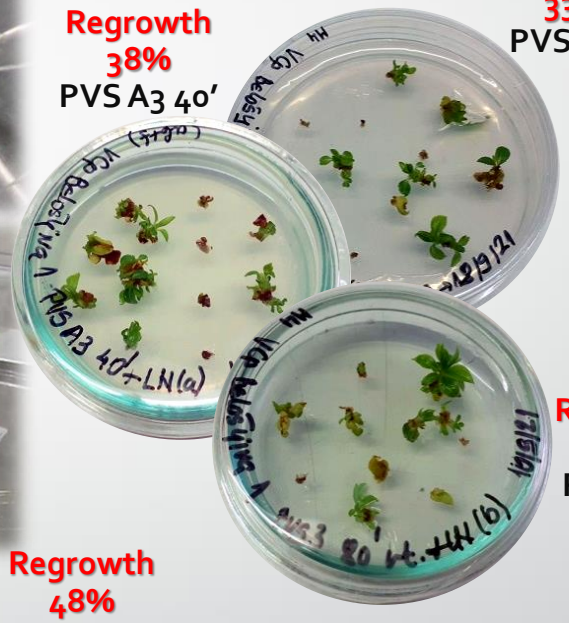


Regrowth 50% PVS A3 20'

Regrowth 52 % PVS A3 40'

Regrowth 67% PVS3 60'

Plum 'Belošljiva'

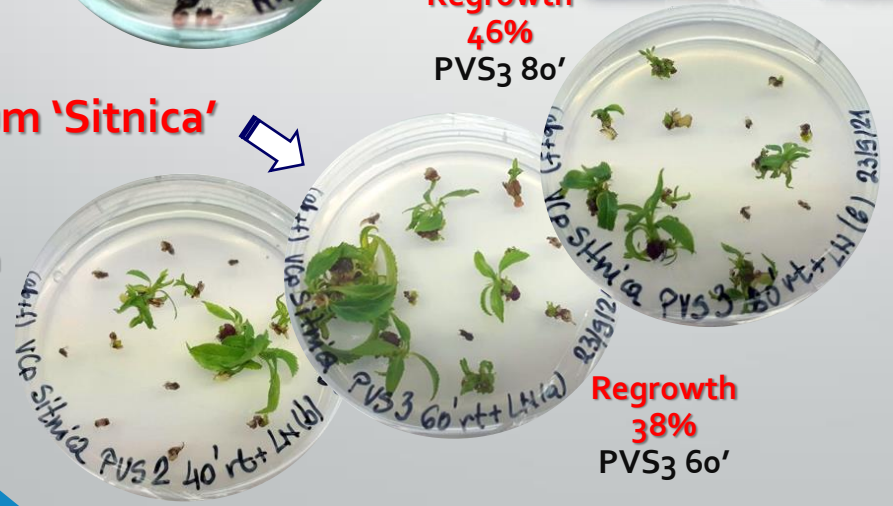


Regrowth 38% PVS A3 40'

Regrowth 33% PVS2 40'

Regrowth 29% PVS3 60'

Plum 'Sitnica'

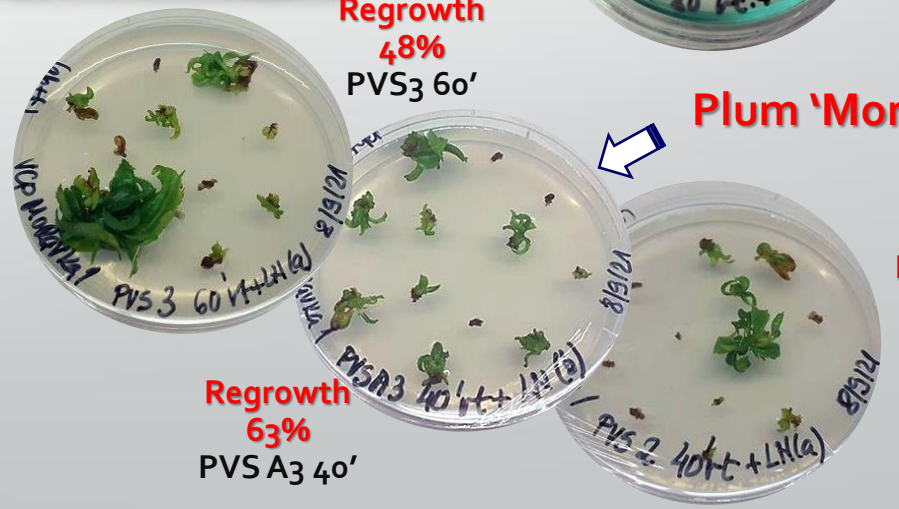


Regrowth 46% PVS3 80'

Regrowth 25% PVS2 40'

Regrowth 38% PVS3 60'

Plum 'Moravka'



Regrowth 48% PVS3 60'

Regrowth 63% PVS A3 40'

Regrowth 21% PVS2 40'

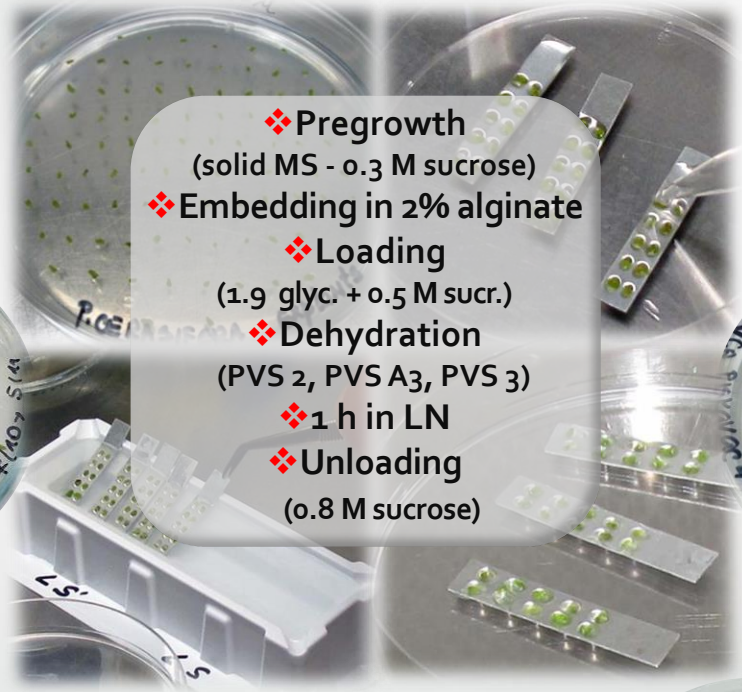
PROMIS Program for Excellent Projects of Young Researchers

Science Fund of the Republic of Serbia

V cryo-plate

CRYOPLUM

- Pregrowth
  (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading
  (1.9 glyc. + 0.5 M sucr.)
- Dehydration
  (PVS 2, PVS A3, PVS 3)
- 1 h in LN
- Unloading
  (0.8 M sucrose)



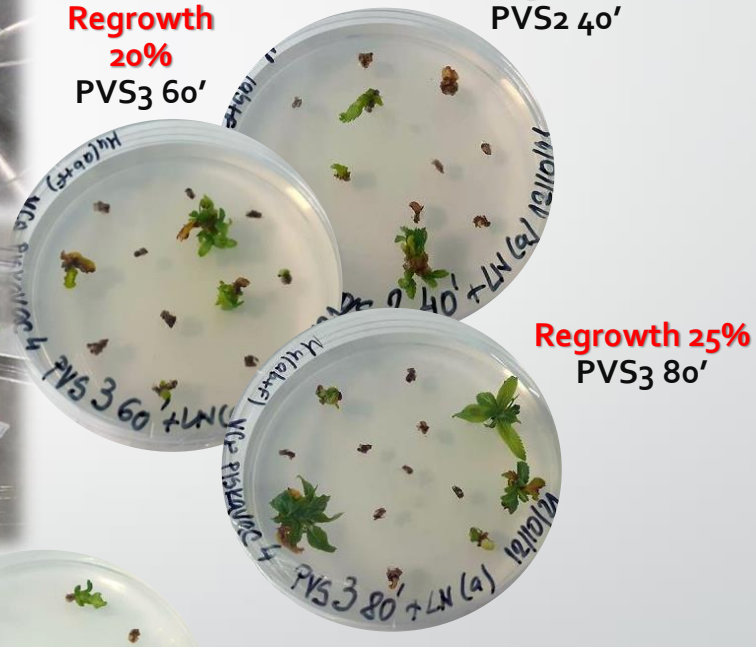
Plum 'Crnošljiva'

Regrowth 30%
PVS A3 20′

Regrowth 43%
PVS3 60′

Regrowth 25%
PVS3 80′

Plum 'Piskavac'

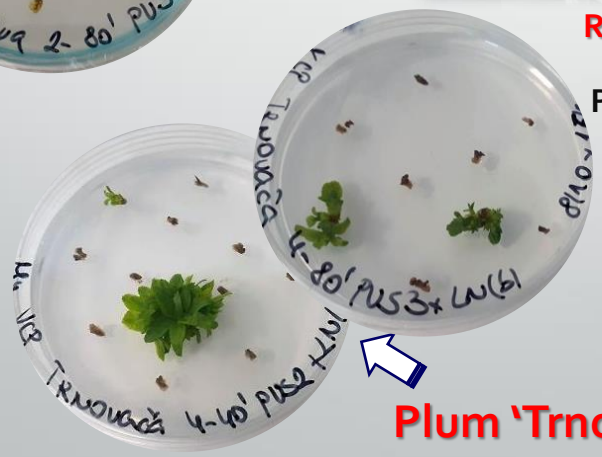


Regrowth 20%
PVS3 60′

Regrowth 30%
PVS2 40′

Regrowth 25%
PVS3 80′

Plum 'Trnovača'



Regrowth 11%
PVS3 80′

Regrowth 8%
PVS2 40′

Plum 'Okruglica'

Regrowth 17%
PVS3 80′

Regrowth 13%
PVS A3 40′

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D cryo-plate

1. Vujović T., Jevremović D., Marjanović T., Ružić Đ. (2021): Cryopreservation of Serbian autochthonous plum 'Crvena Ranka' using aluminium cryo-plates. Genetika, 53, 1: 283–294.

Plum 'Crvena Ranka'

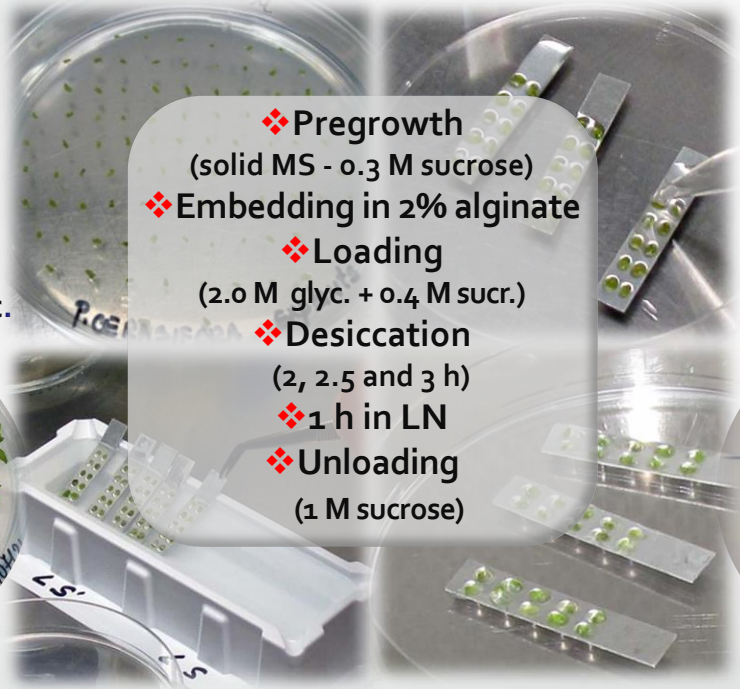
Regrowth 67%
2 h desicc.

Regrowth 42%
2.5 h desicc.

Regrowth 63%
3 h desicc.

CryoPlum

- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0 M glyc. + 0.4 M sucr.)
- Desiccation (2, 2.5 and 3 h)
- 1 h in LN
- Unloading (1 M sucrose)



Plum 'Belošljiva'

Regrowth 42%
2 h desicc.

Regrowth 30%
2.5 h desicc.

Regrowth 33%
3 h desicc.

Plum 'Sitnica'

Regrowth 40%
2.5 h desicc.

Regrowth 42%
2 h desicc.

Regrowth 25%
3 h desicc.

PROMIS
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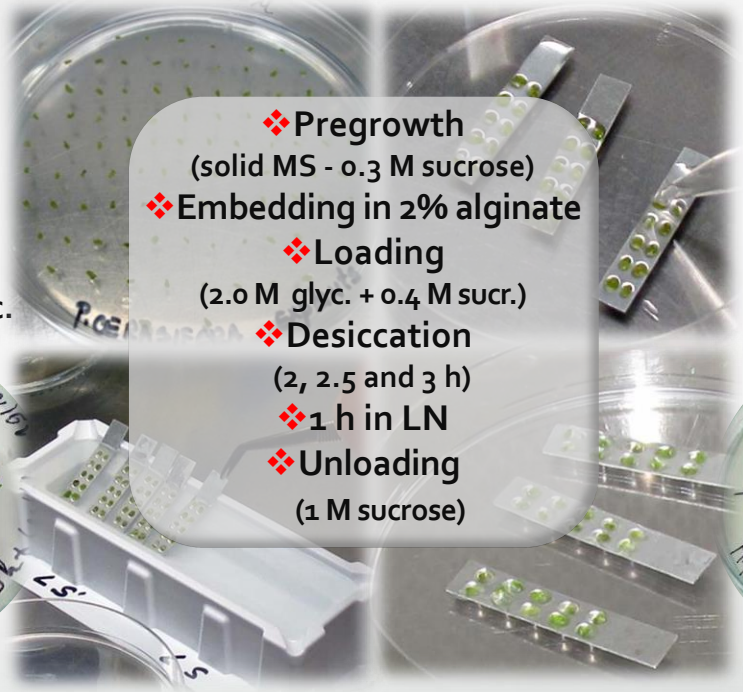
D cryo-plate

CRYOPLUM

- Pregrowth (solid MS - 0.3 M sucrose)
- Embedding in 2% alginate
- Loading (2.0 M glyc. + 0.4 M sucr.)
- Desiccation (2, 2.5 and 3 h)
- 1 h in LN
- Unloading (1 M sucrose)



Plum 'Crnošljiva'

Regrowth 13% — 2 h desicc.

Regrowth 12.5% — 2.5 h desicc.

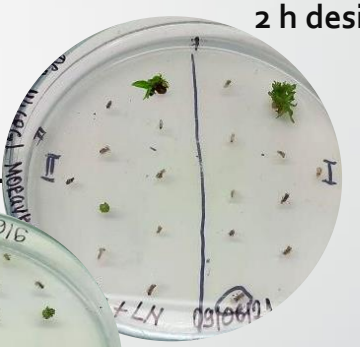
Plums 'Trnovača' and 'Okruglica'

NO regrowth

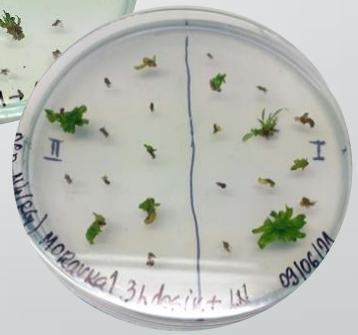
Regrowth 17% — 3 h desicc.

Plum 'Moravka'

Regrowth 10% — 2 h desicc.



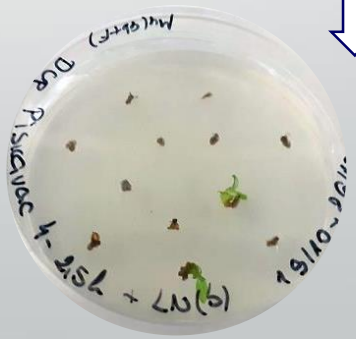
Regrowth 29% — 2.5 h desicc.



Regrowth 25% — 3 h desicc.

Plum 'Piskavac'

Regrowth 8.3% — 2.5 h desicc.



Regrowth 25% — 3 h desicc.

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Cryotherapy

Plant Cell, Tissue and Organ Culture (PCTOC)
https://doi.org/10.1007/s11240-022-02425-y

ORIGINAL ARTICLE

Effect of D and V cryo-plate methods for plum pox virus eradication from two plum cultivars

Darko Jevremović[1] · Bojana Vasilijević[1] · Tatjana Anđelić[1] · Tatjana Vujović[1]

Table 2 Frequency of plum pox virus eradication by cryotherapy in plum 'Belošljiva'

Treatments	Subculture after regrowth				After acclimatization
	1	2	3	4	
LS1 loading control	5/5*	5/5	5/5	5/5	5/5
2 h desiccation − LN	0/5	5/5	5/5	5/5	5/5
2 h desiccation + LN	0/9	9/9	9/9	9/9	9/9
2.5 h desiccation − LN	0/5	5/5	5/5	5/5	5/5
2.5 h desiccation + LN	0/7	1/7	7/7	7/7	7/7
3 h desiccation − LN	0/5	5/5	5/5	5/5	5/5
3 h desiccation + LN	0/12	12/12	12/12	12/12	12/12
C4 loading control	5/5	5/5	5/5	5/5	5/5
PVS2 20 min − LN	0/5	5/5	5/5	5/5	5/5
PVS2 20 min + LN	–	–	–	–	–
PVS2 40 min − LN	0/5	5/5	5/5	5/5	5/5
PVS2 40 min + LN	0/15	13/15	15/15	15/15	15/15
PVS A3 20 min − LN	0/5	5/5	5/5	5/5	5/5
PVS A3 20 min + LN	0/4	3/4	4/4	4/4	4/4
PVS A3 40 min − LN	0/5	5/5	5/5	5/5	5/5
PVS A3 40 min + LN	0/16	16/16	16/16	16/16	16/16
PVS3 60 min − LN	0/5	5/5	5/5	5/5	5/5
PVS3 60 min + LN	0/9	8/9	9/9	9/9	9/9
PVS3 80 min − LN	0/5	5/5	5/5	5/5	5/5
PVS3 80 min + LN	0/19	12/19	19/19	19/19	19/19

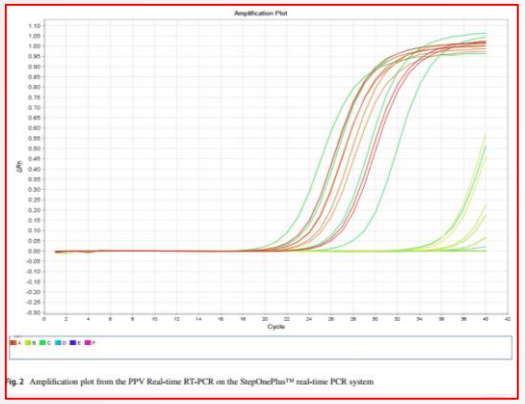

Fig. 2 Amplification plot from the PPV Real-time RT-PCR on the StepOnePlus™ real-time PCR system

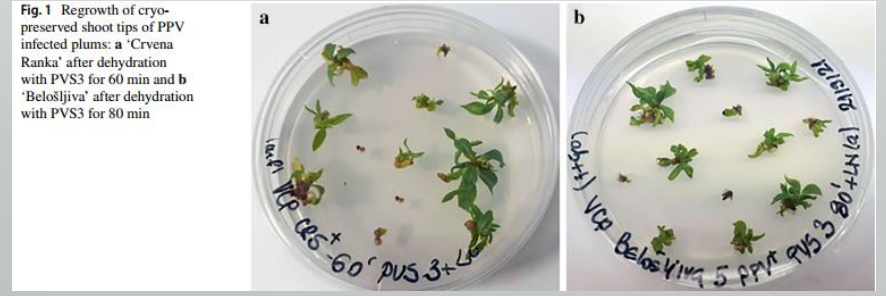

Fig. 1 Regrowth of cryo-preserved shoot tips of PPV infected plums: **a** 'Crvena Ranka' after dehydration with PVS3 for 60 min and **b** 'Belošljiva' after dehydration with PVS3 for 80 min

Plant Cell, Tissue and Organ Culture (PCTOC)

Table 3 Frequency of plum pox virus eradication by cryotherapy in plum 'Crvena Ranka'

Treatments	Subculture after regrowth				After acclimatization
	1	2	3	4	
LS1 loading control	5/5*	5/5	5/5	5/5	5/5
2 h desiccation − LN	5/5	5/5	5/5	5/5	5/5
2 h desiccation + LN	6/9	9/9	9/9	9/9	9/9
2.5 h desiccation − LN	0/5	5/5	5/5	5/5	5/5
2.5 h desiccation + LN	0/6	1/6	3/6	6/6	6/6
3 h desiccation − LN	0/5	5/5	5/5	5/5	5/5
3 h desiccation + LN	0/9	6/9	8/9	9/9	9/9
C4 loading control	5/5	5/5	5/5	5/5	5/5
PVS2 20 min − LN	0/5	5/5	5/5	5/5	5/5
PVS2 20 min + LN	0/4	0/4	0/4	0/4	0/4
PVS2 40 min − LN	0/5	5/5	5/5	5/5	5/5
PVS2 40 min + LN	0/2	0/2	2/2	2/2	2/2
PVSA3 20 min − LN	0/5	5/5	5/5	5/5	5/5
PVSA3 20 min + LN	0/2	0/2	2/2	2/2	2/2
PVSA3 40 min − LN	0/5	3/5	4/5	5/5	5/5
PVSA3 40 min + LN	0/6	0/6	3/6	6/6	6/6
PVS3 60 min − LN	0/5	5/5	5/5	5/5	5/5
PVS3 60 min + LN	0/12	0/12	0/12	0/12	0/12
PVS3 80 min − LN	0/5	4/5	5/5	5/5	5/5
PVS3 80 min + LN	0/6	0/6	0/6	0/6	0/6

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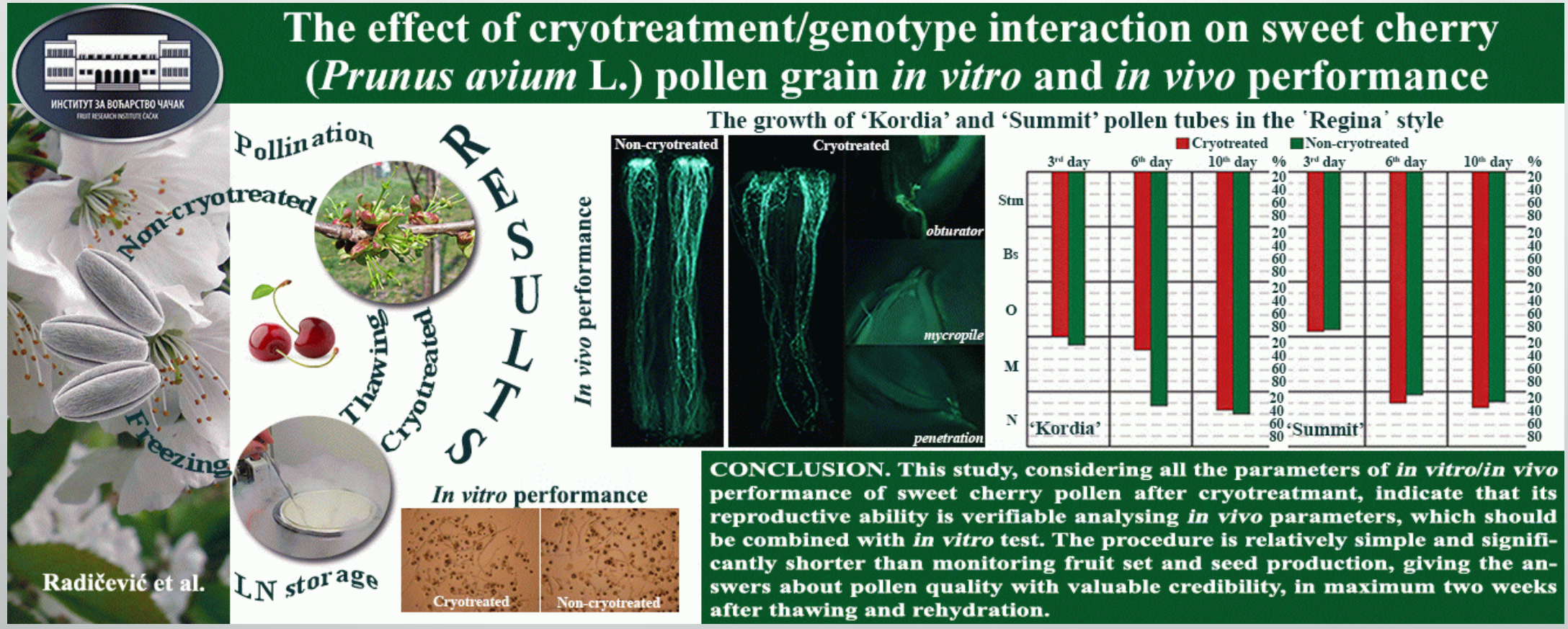
Pollen cryopreservation



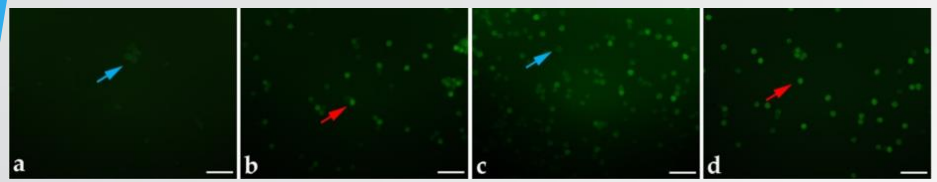
Pollen cryopreservation

horticulturae MDPI

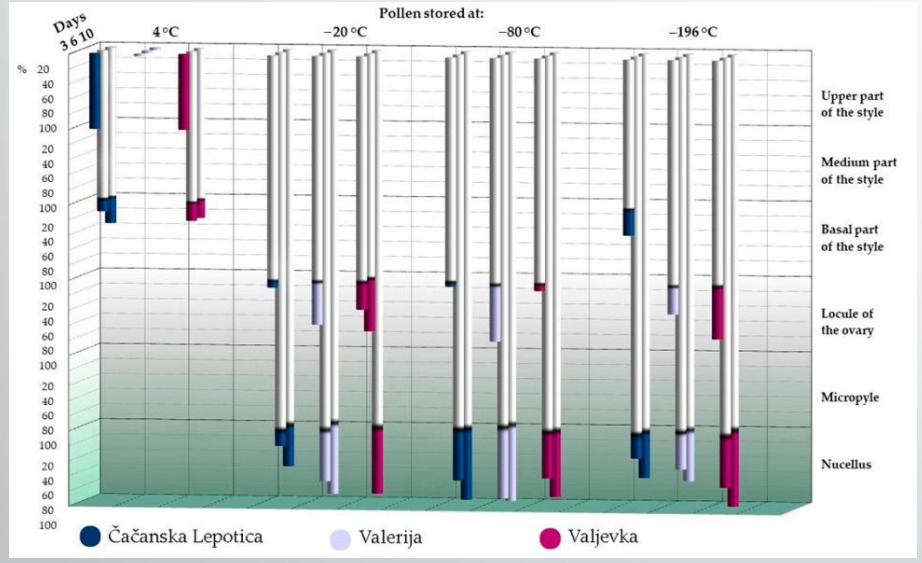
Article

In Vitro and *In Vivo* Performance of Plum (*Prunus domestica* L.) Pollen from the Anthers Stored at Distinct Temperatures for Different Periods

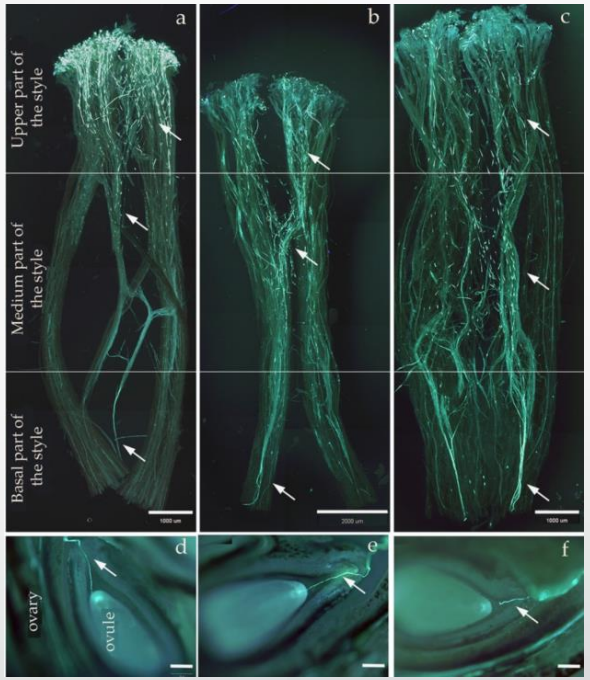
Milena Đorđević [1], Tatjana Vujović [1], Radosav Cerović [2], Ivana Glišić [1], Nebojša Milošević [1], Slađana Marić [1], Sanja Radičević [1], Milica Fotirić Akšić [3] and Mekjell Meland [4,*]



FDA-stained pollen grains after 12 months of storage: (a) at 4 ◦C; (b) at −20 ◦C; (c) −80 ◦C; (d) −196 ◦C. Scale bars = 200 µm. Blue arrows—non-viable pollen grain; red arrows – viable pollen grain

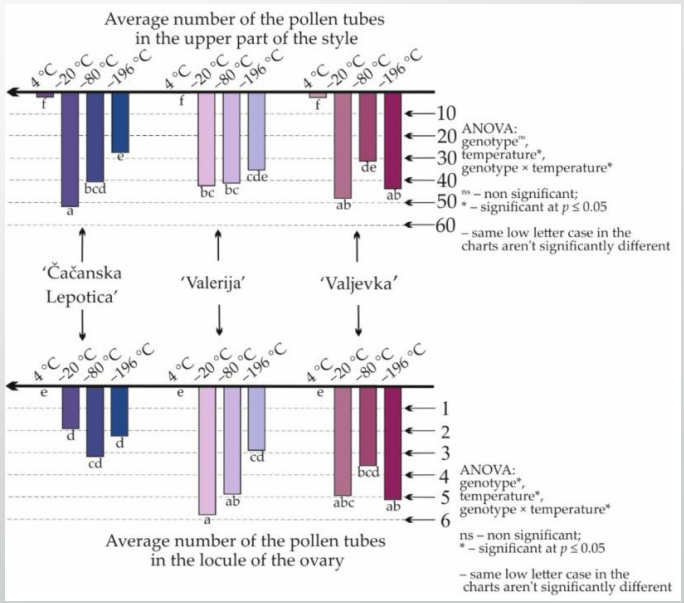


Dynamics of pollen tube growth in the pistils of the Čačanska Lepotica after 12 months of pollen storage at different temperatures.



Growth of pollen tubes into certain parts of the pistils of Čačanska Lepotica from the pollen of: (a,d) Čačanska Lepotica stored at −20◦C; (b,e) Valerija stored at −80◦C; (c,f) Valjevka stored at −196 ◦C. Scale bars: (a,c) 1000 µm; (b) 2000 µm; (d,e,f) 200 µm. Arrows indicate pollen tubes.

Average number of pollen tubes in certain parts of the pistil of Čačanska Lepotica



What we have not done so far

- We did not establish cryo-bank of temperate fruit species in Serbia because up to now funding was limited to research projects on cryopreservation.

Further steps

- Fruit Research Institute will launch an initiative for the formation of a national cryo bank for fruit species. The establishment of the bank should be under the authority of the Ministry of Agriculture, Forestry and Water Management.

Idea

- Large European project on cryopreservation with the aim to form a centralized European cryo bank for fruit species. Each partner to develop a protocol for their most important autochthonous genotypes and deposit specimens into the bank.


Thank you for
your attention
ИНСТИТУТ ЗА ВОЋАРСТВО ЧАЧАК
FRUIT RESEARCH INSTITUTE ČAČAK