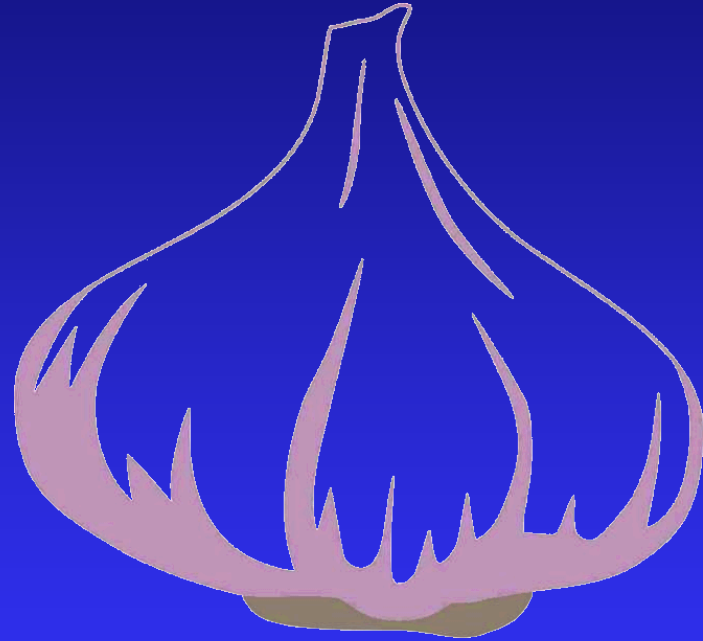


# Cryopreservation - state of the art for vegetatively propagated *Alliums*



Miloš Faltus

Plant Physiology and Cryobiology Lab

Crop Research Institute - Prague

# PLANT PHYSIOLOGY AND CRYOBIOLOGY TEAM

Diana Däppenová

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Jiří Zámečník

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Miloš Faltus

Zdeňka Hybnerová

Petra Šimůnková



Crop Research Institute - Prague



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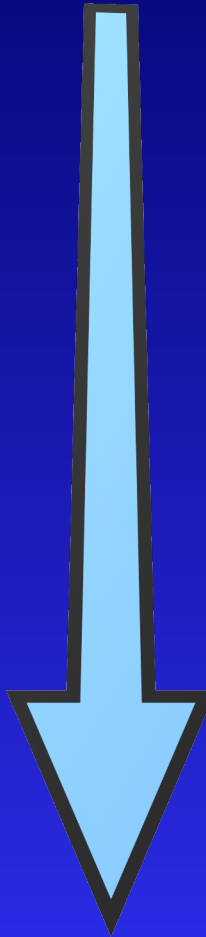
2018 PRAGUE SLTB meeting

2021 Cryobank reconstruction

2022 Cryopreservation WG of ECPGR establishment



*45 years of cryobiology study, 20 years of the cryobank operation*





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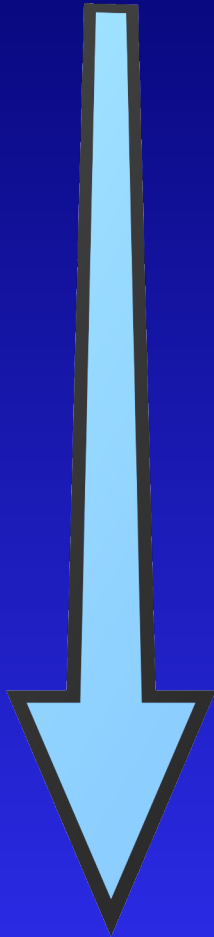
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# Strategy of cryoconservation in the Czech Republic

## National Program for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity

### *Basic strategy of plant germplasm cryoconservation*

- safety duplication of basic collections (different storage method and locality)
- keeping the most valuable genetic material of Czech origin

### *National curators of crop germplasm of vegetatively propagated crops:*

Research and Breeding Institute of Pomology Holovousy – **temperate fluit trees**

MENDELU Lednice – **thermophilic temperate fluit trees**

Potato research Institute Havlíčkův Brod – **potato** (*in vitro*)

Hop Research Institute Žatec – **hop**

VSV Karštejn CRI, Ampelos Vrbovec, MENDELU Lednice – **Vitis**

CRI Olomouc - **Allium**



**Central cryobank** in CRI Prague – collaboration with plant germplasm curators, that provide the most valuable samples for their backup.

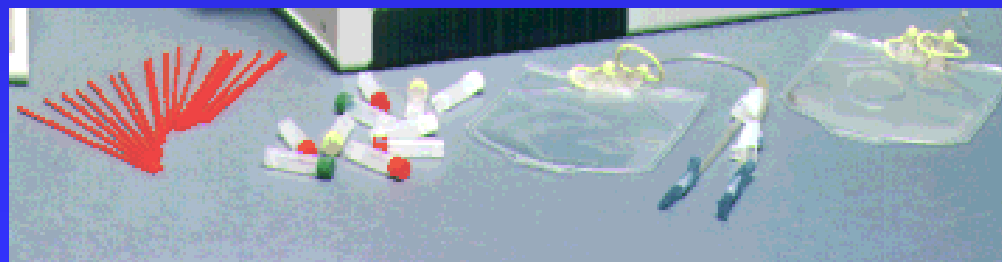
# Strategy of cryoconservation in the Czech Republic

Species	Number of cryopreserved accessions in the cryobank
<i>Malus domestica</i> BORKH.	17
<i>Pyrus communis</i> L. (E	24
<i>Prunus armeniaca</i> L.	12
<i>Cerasus avium</i> (L.) M	3
<i>Cerasus vulgaris</i> P.M	10
<i>Cerasus</i> P.MILLER (ot	3
<i>Fragaria x ananassa</i>	34
<i>Lonicera</i> L. (edible	24
<b><u>Allium sativum</u> L.</b>	<b><u>187</u></b>
<i>Solanum tuberosum</i> L1	104
<i>Vitis vinifera</i> L.	3
<i>Malus</i> MILL. <hort. c	6
<i>Humulus lupulus</i> L.	70
<i>Prunus persica</i> var. <i>Persica</i>	5
<b>Total</b>	<b>502</b>

# Strategy of cryoconservation in the Czech Republic

## Cryobank Storage Technology

- in Dewar flask
- cryovials
- barcode



# Strategy of cryoconservation in the Czech Republic

## CRYOPRESERVATION

- a **method for long-term storage** of living organisms /or of their parts/ at ultra-low temperature
- **principle** – glassy state of matter with characteristics of a solid state without any significant changes during storing
- **the glassy state** is made possible by previous massive dehydration

Two basic approaches (with respect to dehydration and way of cooling):

- **Freezing** – controlled ice crystal growth (freezing dehydration)
- **Vitrification** – avoidance of ice crystal growth
  - *Air-dehydration* (encapsulation-dehydration)
  - *Osmotic dehydration* (vitrification method)



Zámečník Faltus Bilavčík 2007, Adv Hort Sci 21,247



# Allium cryopreservation

Cloves

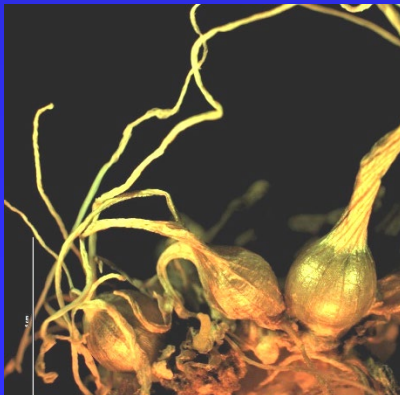


Bulbils

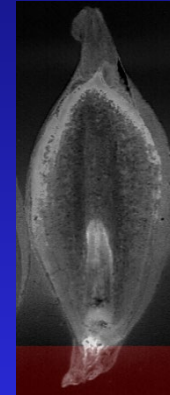
Bolting garlic  
only



*in vitro*



Top sets

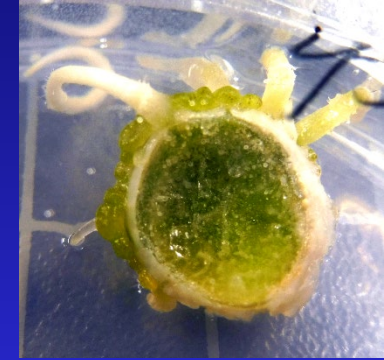


ripe

unripe



Basal  
plate

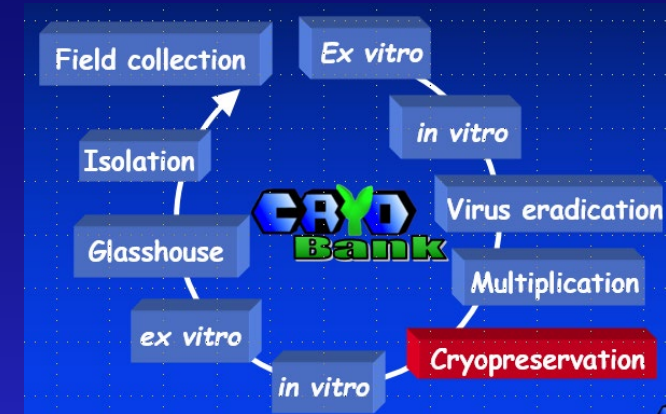


Lynch, P. T., Souch, G. R., Zámečník, J., & Harding, K. (2016). Optimization of water content for the cryopreservation of *Allium sativum* *in vitro* cultures by encapsulation-dehydration. *CryoLetters*, 37(5), 308-317

# Allium cryopreservation

## PVSx method development

- 1991 PVS2 Sakai - first used PVS2
- 1995 Niwata - first used PVS2 for garlic
- 1999 Makowska **PVS3**>PVS2
- 2003 Zámečník Czech Garlic Cryobank **PVS3** – only
- 2003 Keller **PVS3**
- 2003 Kim PVS2
- 2004 Volk PVS2
- 2005 Kim **PVS3** > PVS2 > PVS1 > Fahy > Steponkus
- 2006 Volk PVS2
- 2007 EURALLIVEG **PVS3** only
- 2009 Kim **PVS3** > PVS2
- 2011 Kim **PVS3** > 12 PVSx
- 2011 Tripartitate Cryobank **PVS3** only
- 2017 Liu **PVS3**

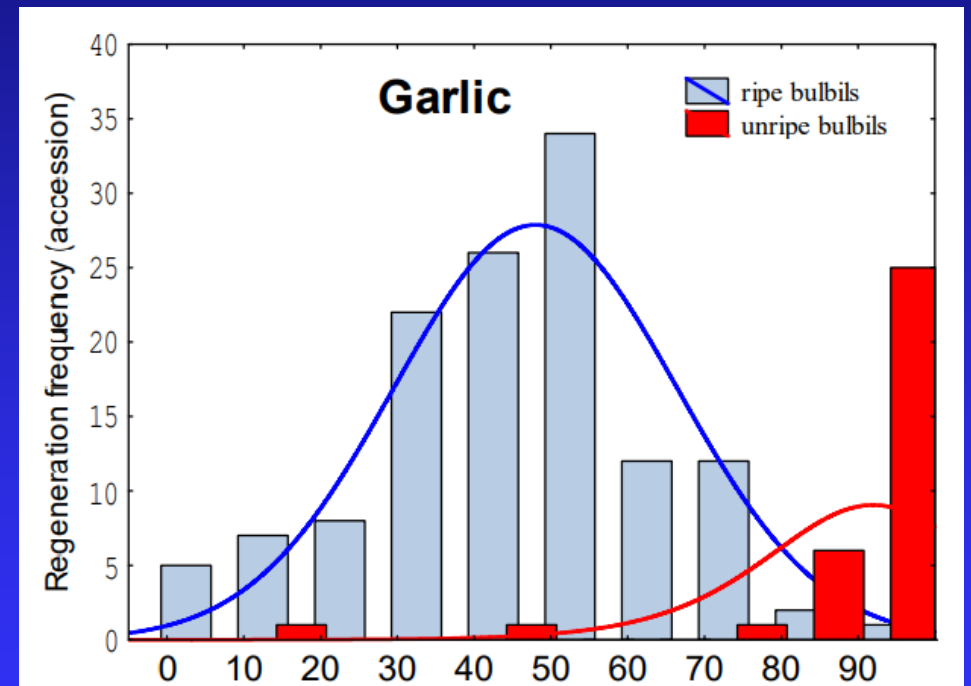
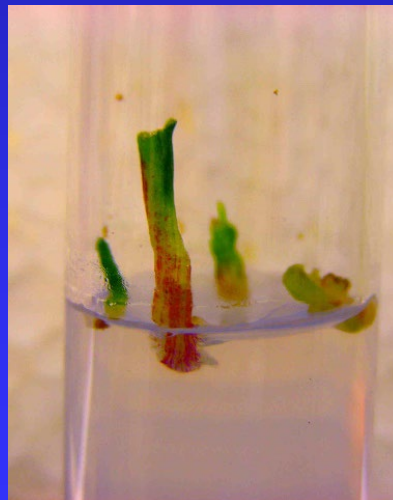


# Allium cryopreservation

## Efficiency of the cryopreservation method

### Cryoprotocol Steps

- Loading solution sucrose + glycerol
- Dehydration by PVS3 - 2 hours
- Aluminum foil 10 shoot tips
- Plunged into LN
- Rapid warming 40 °C
- Survival and regeneration evaluation



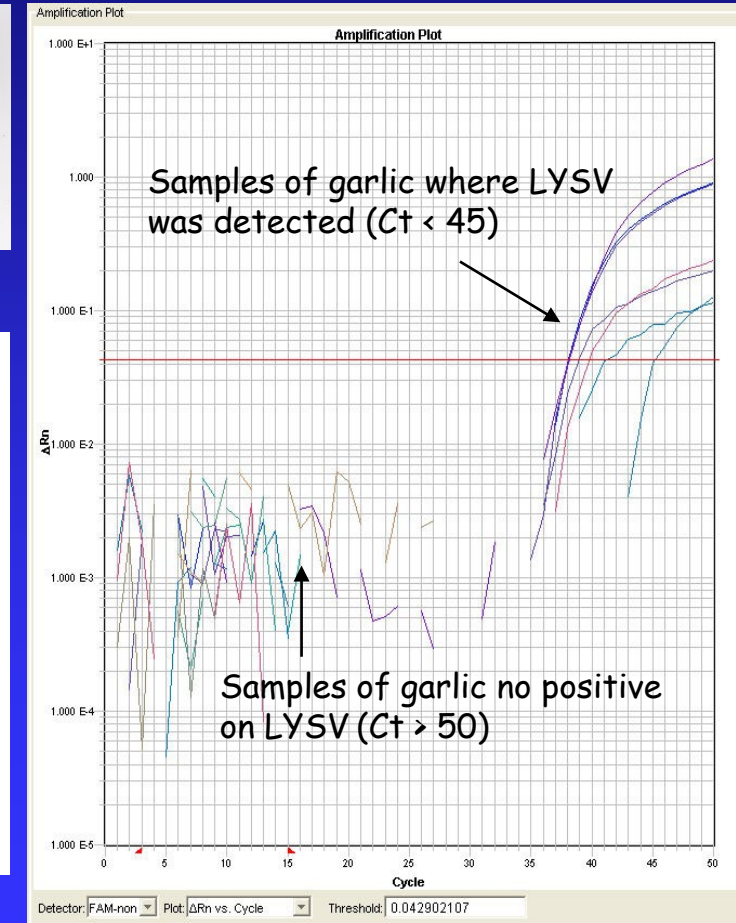
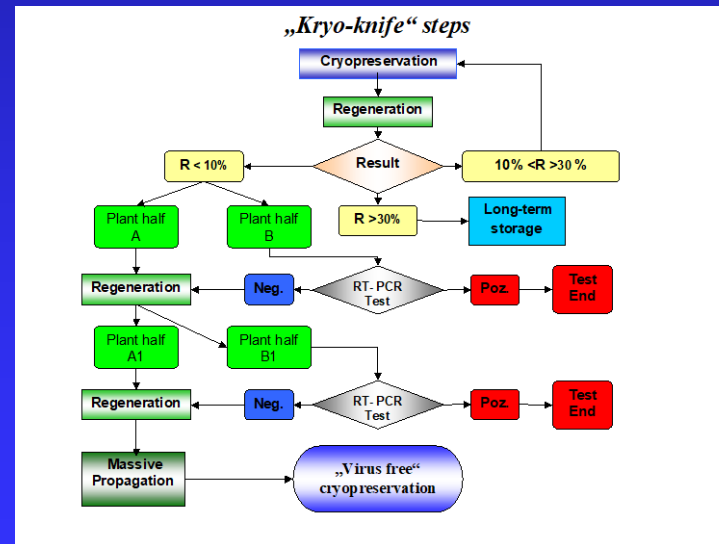
Zámečník et al., 2012, in *Cryopreservation, Intech*, 333-358

# Allium cryopreservation

## Cryotherapy „Cryo-knife“

## Real time RT PCR

	1st run	OYDV	GCLV	LYSV	SLV		2nd run	OYDV	GCLV	LYSV	SLV
4x	1309 NK	+	+	+	+						
	1309/2 PK	+	+	+	+		1309/2/PK	+	+	+	+
	1309/3 PK	-	+	+	+						
	1309/1 PK	-	-	+	-		1309/1/PK A	-	-	+	-
							1309/1/PK B	-	-	+	-
2x	1394 C	-	-	+	+						
	1394/1 PK	-	-	-	-		1394/1/PK A	-	-	-	-
							1394/1/PK B	-	-	-	-
							1394/1/PK C	-	-	-	-
							1394/1/PK D	-	-	-	-
	1394/2 PK	-	-	-	-		1394/2/PK	-	-	-	-
	1394/3 PK*	-	-	-	-		1394/3/PK	-	-	-	-
1x	1325 NK	-	-	-	+						
	1325PK*	-	-	-	-		1325/1/PK	-	-	-	-
							1325/2/PK	-	-	-	-
							1325/3/PK	-	-	-	-
0x	1229 NK*	-	-	-	-		1229/1/NK	-	-	-	-
							1229/2/NK	-	-	-	-
		1229/1 PK	-	-	-	-		1229/1/PK A	-	-	-
								1229/1/PK B	-	-	-
		1229/2 PK*	-	-	-			1229/2/PK	-	-	-
	1669/1/PK	-	-	-	-						
	1669/2/PK	-	-	-	-						
	1669/3/PK	-	-	-	-						
	1669/4/PK A	-	-	-	-						
	1669/4/PK B	-	-	-	-						
	1669/5/PK	-	-	-	-						
	1669/6/PK	-	-	-	-						
	1669/7/PK	-	-	-	-						
	1669/8/PK	-	-	-	-						
	1669/9/PK	-	-	-	-						
	1669/10/PK	-	-	-	-						

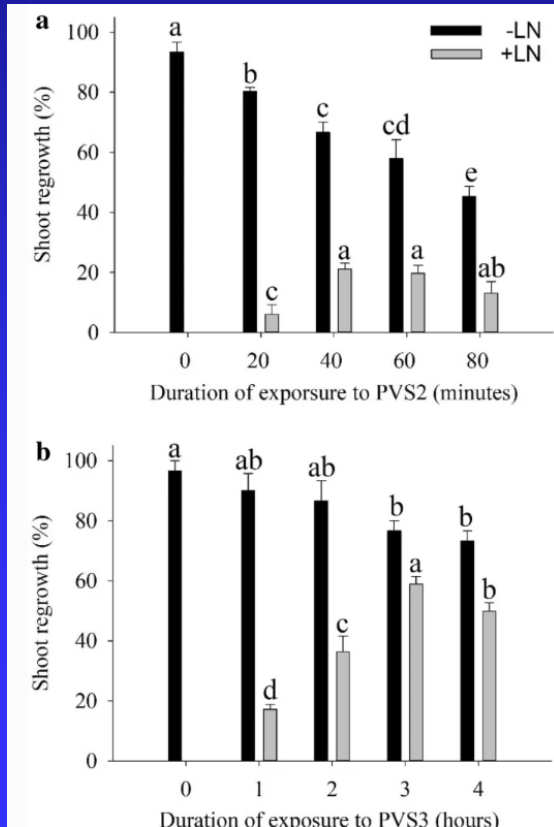


# Allium cryopreservation

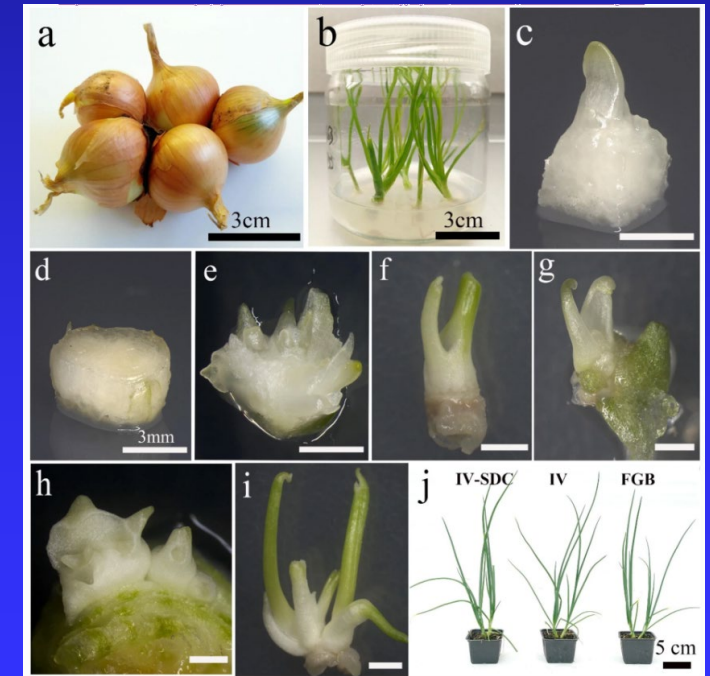
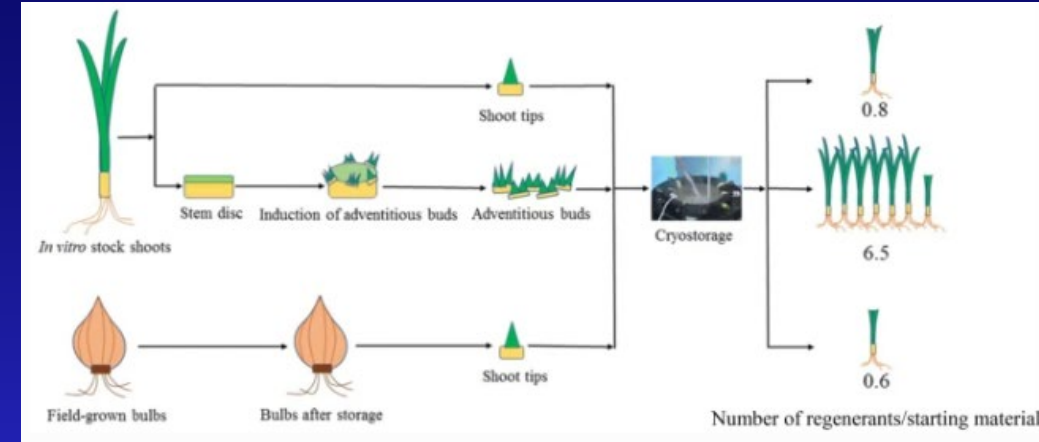
## Cryopreservation of Shallot

Background – Shallot cryopreservation  
EURALLIVEG

The same story PVS3 > PVS2

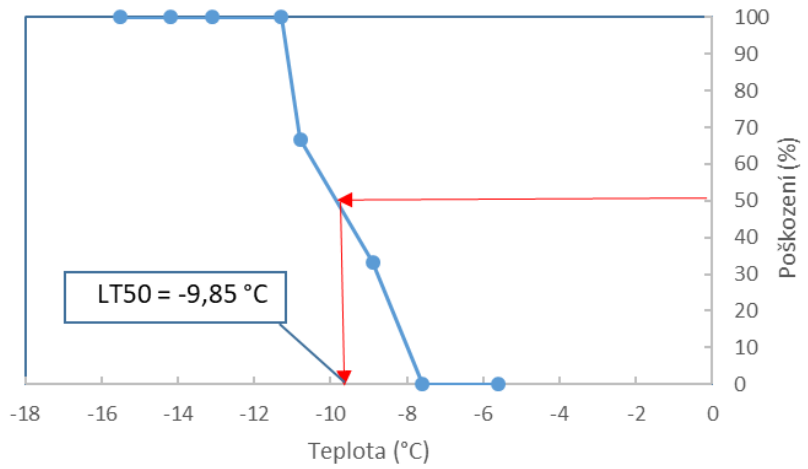


Wang, M. R., Zhang, Z., Zámečník, J., Bilavčík, A., Blystad, D. R., Haugslie, S., & Wang, Q. C. (2020). Droplet-vitrification for shoot tip cryopreservation of shallot (*Allium cepa* var. *aggregatum*): Effects of PVS3 and PVS2 on shoot regrowth. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 140(1), 185-195.

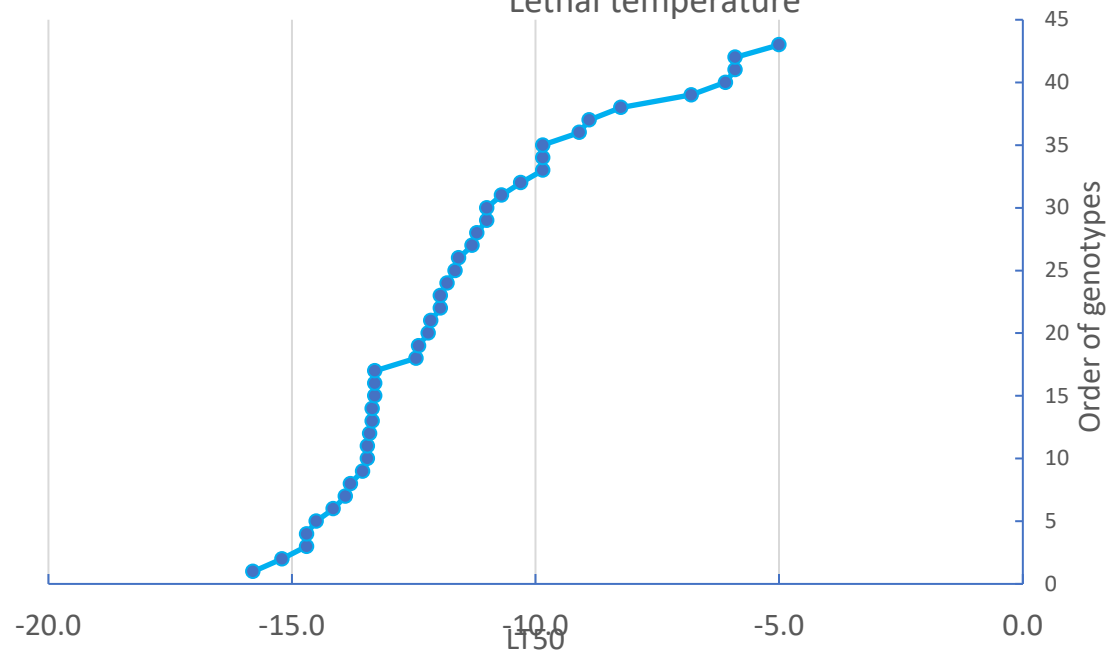


# Allium frost resistance tests

Příklad teplotního zásahu (genotyp 37)



Lethal temperature

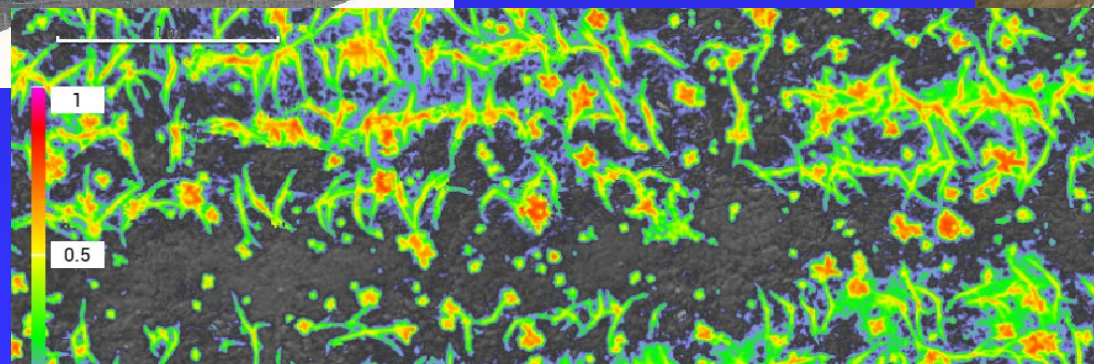
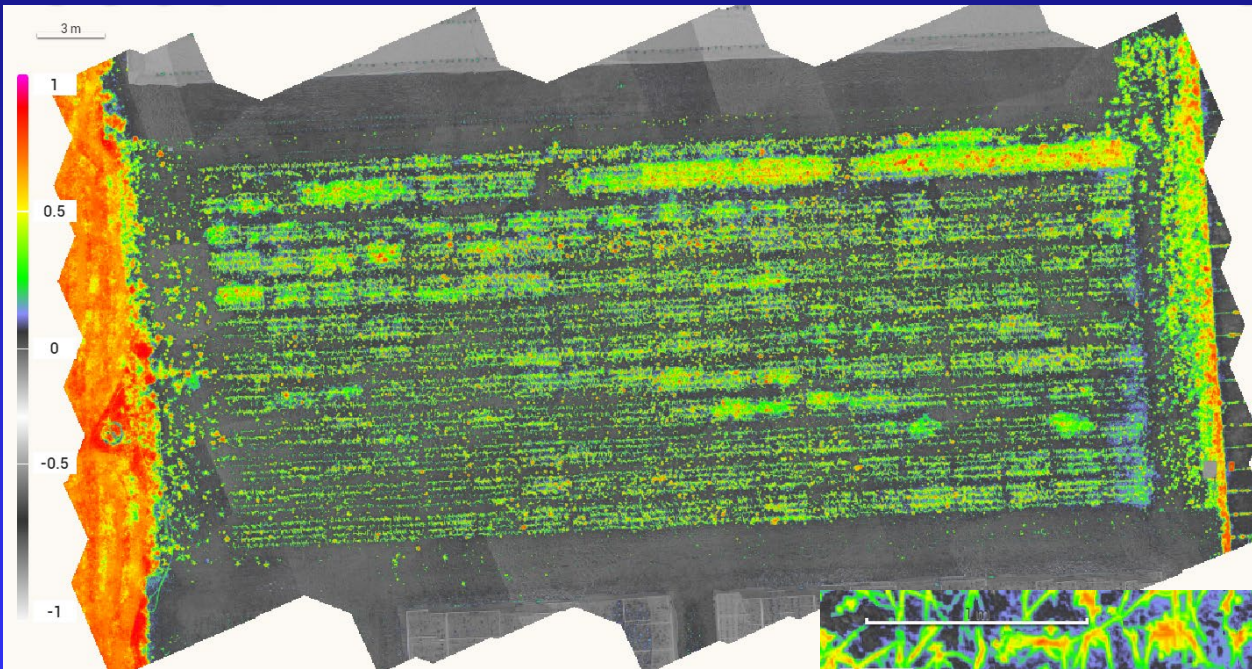


# Allium drought resistance evaluation

## Remote sensing of garlic genotypes

NDVI Normalized Difference Vegetation Index

$$\text{NDVI} = \frac{\text{NIR} - \text{RED}}{\text{NIR} + \text{RED}}$$



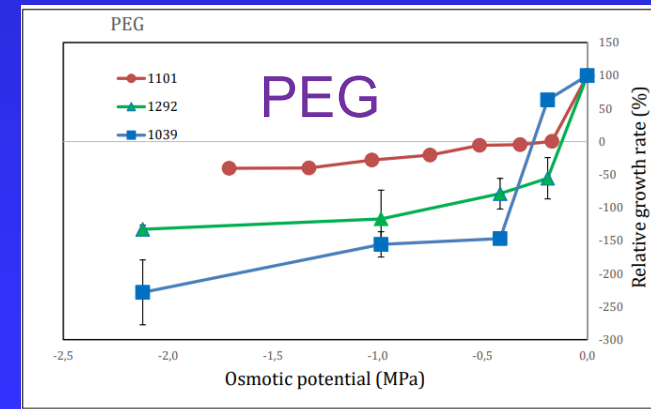
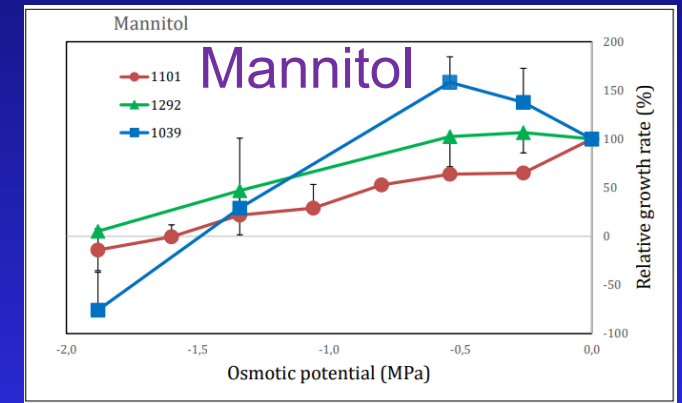
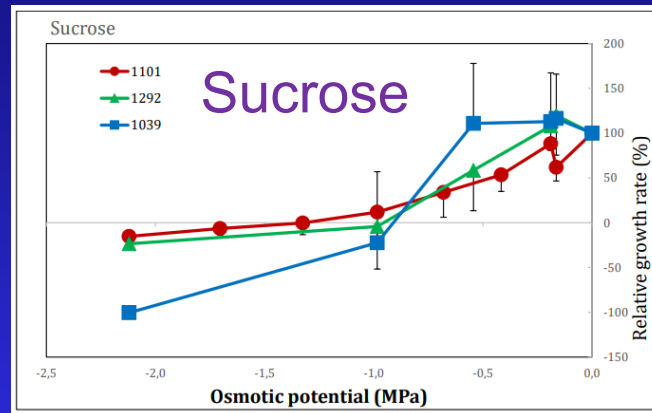
# Allium drought resistance evaluation

## In vitro Drought Resistance Test



$P_n \sim 0$   
 $I_T \sim 0$   
 $T = \text{const.}$   
 $P_p = \text{const.}$

Direct physiological reaction to insufficient water intake, simulated by increased osmotic potential.







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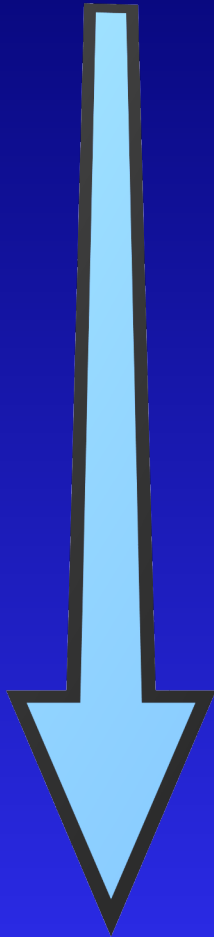
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*45 years of cryobiology study, 20 years of the cryobank operation*



# Involvement in EU cooperation in the field of cryoconservation

2005 CRYMCEPT workshops

Leuven



Montpellier



Eighth Meeting of the *Allium* Working Group, 11-12 October 2022, Skierniewice, Poland



# Involvement in EU cooperation in the field of cryoconservation

2007 – 2011 CRYOPLANET – COST ACTION 871

Oviedo, Spain

Angers, France



# Involvement in EU cooperation in the field of cryoconservation

## TA SCHOOL 2007 – 2009 (under the COST ACTION 871)



# Involvement in EU cooperation in the field of cryoconservation

2009 – 2011 EURALLIVEG – a pilot European project on garlic cryopreservation



Eighth Meeting of the *Allium* Working Group, 11-12 October 2022, Skierniewice, Poland



# Involvement in EU cooperation in the field of cryoconservation

In April 2007, an European project named EURALLIVEG (EUROpean ALLium germplasm VEGetatively maintained) started under Council Regulation EC 870/2004



- Dr. E. R. Joachim Keller Dr. Christine Zanke GERMANY
- Dr. Jiří Zámečník Dr. Helena Stavělíková CZECH REPUBLIC
- Dr. Teresa Kotlinska; Marta Olas-Sochacka POLAND
- Prof. Dr. Vito Miccolis; Luciana Altieri ITALY
- Dr. Chris Kik THE NETHERLANDS
- Florence Esnault FRANCE
- NordGen, SWEDEN

Garlic was cryopreserved by three project participants

- IPK Dr. Dr. E. R. Joachim Keller - coordinator
- CRI Dr. Jiří Zámečník - cryopreservation response
- RIVC Dr. Teresa Kotlinska - cryopreservation

The final objective was to establish a **Tripartite Cryopreservation Genebank.**

Europe's garlic germplasm maintenance supervised and coordinated by the European Cooperative Programme for Crop Genetic Resources **ECPGR**

# Involvement in EU cooperation in the field of cryoconservation

## Cryopreservation of Garlic for the Establishment of an European core Collection

- Vitrification method with the PVS3 - 200 most important garlic accessions
- The weighted average survival for these cryopreserved accessions was 72% and the regrowth was 50%.

Participants of EURALLIVEG of the first and the final meeting

2007: IPK, Gatersleben

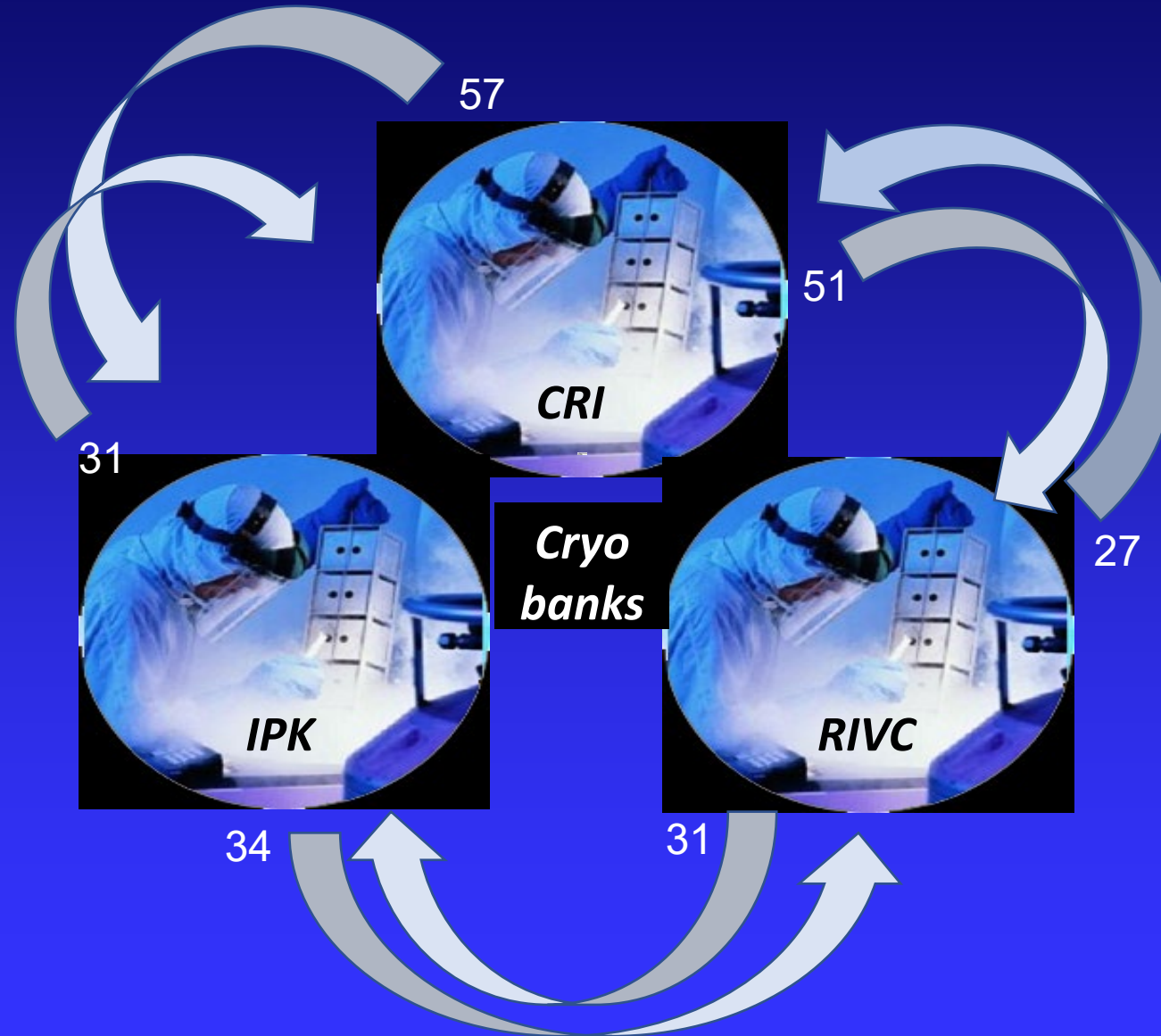


2011: CRI, Prague



Zanke, C., Zamecnik, J., Kotlińska, T., Olas, M. and Keller, E.R.J. (2011). CRYOPRESERVATION OF GARLIC FOR THE ESTABLISHMENT OF A EUROPEAN CORE COLLECTION. Acta Hort. 908, 431-438

# Involvement in EU cooperation in the field of cryoconservation



Number of Exchange  
Secure Duplicate  
Accessions



# Involvement in EU cooperation in the field of cryoconservation

## The ECPGR Working Group on Cryopreservation

- established in February 2022 based on proposal of Dr. Nicolas Roux, Dr. Bart Panis, Dr. Manuela Nagel, Dr. Stephane Dussert
- ECPGR Executive Committee nominated Dr. Bart Panis and Dr. Miloš Faltus as co-Chairs of the ECPGR Cryopreservation Working Group in August 2022
- 15 countries, 26 members, 4 curators, 3 crop specialists

### Current issues:

- There is no extensive and reliable cryobank infrastructure in the EU
- Backups of crop collections are not coordinated
- Cryopreservation methods are not always available for routine use – personnel with specific skills are required, transfer of cryoprotocols is not easy

Country	Name	Organization	Contact Details	Expertise	Remarks
Albania	Ms Antoneta Brahushi	Institute of Plant Genetic Resources of Albania, Agricultural University of Tirana  Albania	Tel: (355) 69705 2084 ✉ antonetabrahushi@yahoo.com	Cryopreservation expert	
Belgium	Mr Bart Panis Co-chair	The Alliance of Bioversity International and CIAT, c/o KU Leuven Willem de Croylaan 42 bus 2455Kardinaal Mercierlaan 92 3001 Leuven Belgium	Tel: (32-16) 321690 Fax: (32-16) 321993 ✉ b.panis@cgiar.org; bart.panis@biw.kuleuven.be	Cryopreservation expert	
Bulgaria	Ms Stanislava Stateva	Institute for Plant Genetic Resources Druzha str. 2 4122 Sadovo Bulgaria	✉ stanislava.stateva@gmail.com	Cryopreservation expert	
Bulgaria	Ms Katya Uzundzhaliyeva	Institute for Plant Genetic Resources Druzha str. 2 4122 Sadovo Bulgaria	Tel: (359) 32 629026 ✉ kspasova69@gmail.com	Cryopreservation expert	
Czech Republic	Mr Alois Bilavcik	Výzkumný ústav rostlinné výroby, v.v.i. Praha (VÚRV) Crop Research Institute, Cryobank Drnovská 507 161 06 Praha 6 - Ruzyne	✉ bilavcik@vurv.cz	Cryopreservation expert (fruit trees)	
Czech Republic	Mr Milos Faltus Co-chair Contact person	Výzkumný ústav rostlinné výroby, v.v.i. Praha (VÚRV) Crop Research Institute  161 06 Prague, Ruzyne 507 Czech Republic	Tel: (42) 2 33022362 ✉ faltus@vurv.cz	Genebank curator	
Czech Republic	Jiří Zámečník	Výzkumný ústav rostlinné výroby, v.v.i. Praha (VÚRV) Crop Research Institute, Plant Physiology and Cryobiology Team Drnovská 507 161 06 Praha 6 - Ruzyne Czech Republic	Tel: (420) 233022426 Fax: (420) 233022286 ✉ zamecnik@vurv.cz	Cryopreservation expert (garlic)	
Finland	Ms Saija Rantala	Natural Resources Institute Finland (Luke), Production systems, Horticulture technologies Survontie 9 FI 40500 Jyväskylä Finland	Tel: (358) 295326465 ✉ saija.rantala@luke.fi		Cryopreservation expert
France	Mr Philippe Chatelet Contact person	INRAE Bâtiment Arcad, 10 avenue Arthur Young 34090 Montpellier France	Tel: (33) 04 32722201 ✉ philippe.chatelet@inrae.fr		Plant cryopreservation (vegetatively propagated species)
France	Mr Stéphane Dussert	IRD, UMR Diade 911 Av Agropolis 34394 Montpellier France	Tel: (33) 04 67416459 ✉ stephane.dussert@ird.fr		Plant cryopreservation (non-orthodox seeds)
France	Ms Agnès Grapin	Institut Agro Rennes-Angers 2, rue André Le Nôtre 49045 Angers Cedex 01 France	Tel: (33) 02 41225490 ✉ Agnes.Grapin@agrocampus-ouest.fr		Plant cryopreservation (shoot-apex)
Germany	Ms Monika Höfer	Julius Kühn-Institut (JKI) - Federal Research Centre for Cultivated Plants Institut für Züchtungsforschung an gartenbaulichen Kulturen und Obst Pillnitzer Platz 3a 01326 Dresden Germany	Tel: (49) (0) 3946 47 8009 Fax: (49) (0) 3946 47 8002 ✉ monika.hoefer@julius-kuehn.de		Cryopreservation expert
Germany	Ms Manuela Nagel	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) OT Gatersleben 06466 Stadt Seeland Germany	Tel: (49) (0)39482 5156 ✉ nagel@ipk-gatersleben.de		Cryopreservation expert
Germany	Mr Franco Röckel	Julius Kühn-Institut (JKI), Federal Research Institute for Cultivated Plants, Institut für Grapevine breeding Geilweilerhof  76833 Siebeldingen Germany	Tel: (49) (0) 634541216 ✉ franco.roeckel@julius-kuehn.de		Cryopreservation expert

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# Involvement in EU cooperation in the field of cryoconservation

## The ECPGR Working Group on Cryopreservation

### Objectives – areas of interests:

- long-term storage **facilities** and experienced cryopreservation **researchers**
- advanced plant cryopreservation **research** in Europe
- available cryopreservation technologies for germplasm of species that produce **non-orthodox seeds**
- possibility for cryopreserving **orthodox seed collections** (short-lived seeds as leeks, onions and parsley)
- cryopreservation to crop **wild relatives or wild species** and trees
- **collaboration** between European scientists and institutes holding crop collections
- new biobank **technologies and data management systems** for cryopreserved collections

# Involvement in EU cooperation in the field of cryoconservation

## The ECPGR Working Group on Cryopreservation

### Main goals:

#### 1) NETWORKING

Identification of partners involved in plant cryoconservation in EU, facilities, crops, staff  
National coordinators involving  
Sharing of information

#### 2) RESEARCH IMPROVEMENT

Cryopreservation methods standardization  
Information/experience exchange between researchers and cryobanks  
Introduction of new cryoprotocols into cryobanks

#### 3) STRATEGY OF CONSERVATION

Methodology (in vitro, ex vitro, methods, sample size, controls, recovery, ...)  
Facilities - standardization  
Prioritization – species, genotypes

## The ECPGR Working Group on Cryopreservation

### Current action:

Acquisition of information about the current status of cryopreservation in collaboration with national contact persons ECPGR and the ECPGR WG leaders.

Identification of all national groups among EU

1. National cryo-facilities (national cryobanks)
2. Cryoconserved plant species (list of crops)
3. Cryopreservation methods used (freezing/vitrification, explant preculture, procedure for recovery)
4. National strategy for cryoconservation (national crop priority, safety backup management, numbers of stored individuals for accession, control sample size and recovery,..)

Thank you for your attention!