



ECPGR Cryopreservation Working Group

1st Meeting of the ECPGR Cryopreservation Working Group

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



ECPGR Cryopreservation Working Group

Rationale

- world's plant species are threatened
- field collections are not always suitable because germplasm can be lost due to pests, diseases and adverse weather conditions

There is a need of the safety backup collection – cryopreservation is the best option:

- genetically stable conditions
- limited risks for mutations
- low costs
- disease- and pest-free conditions



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Rationale

However, CRYOPRESERVATION is still not a standard, routinely used option for plant germplasm conservation.

Limits:

- lack of long-term experience
- specific lab conditions
- specific crop/genotype sensitivity



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- specific lab conditions
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Limits can be overcome by:

- sharing experience
- skills training
- setting standards



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Rationale

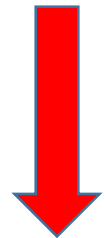
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ECPGR CRYO WG establishment



ECPGR Cryopreservation Working Group

OBJECTIVES OF ECPGR FOR PHASE X (2019-2023)

(agreed at the 15th Steering Committee meeting, May 2018)



LONG-TERM GOAL

Stakeholders in Europe collaboratively, rationally and effectively conserve *ex situ* and *in situ* PGRFA, provide access and increase sustainable use.

OBJECTIVES

click on the 'objective' below to go to the respective page

1	To efficiently conserve and provide access to unique germplasm in Europe through AEGIS and the European Collection
2	To provide passport and phenotypic information of actively conserved European PGRFA diversity <i>ex situ</i> and <i>in situ</i> through the EURISCO catalogue
3	To improve <i>in situ</i> conservation and use of crop wild relatives
4	To promote on-farm conservation and management of European PGRFA diversity
5	To promote use of PGRFA



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**Efficiency of plant
germplasm
conservation increase**



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CRYOCONSERVATION



**Efficiency of plant
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ECPGR Cryopreservation Working Group

Outputs	Activities	Responsibility	Indicators	Assumptions
1.5 Options and opportunities for a cryopreservation network explored	1.5.1 Organize a meeting to identify cryopreservation needs and aims and consider setting up a dedicated network	1.5.1 Relevant WG members; Secretariat	1.5.1.1 Recommendations published; Framework for a cryopreservation network defined 1.5.1.2 Number of vegetatively propagated accessions cryopreserved	



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NETWORKING

+

NEEDS IDENTIFICATION

=

RECOMMENDATIONS PUBLISHED

ACCESSIONS CRYOPRESERVED

CRYOPRESERVATION FRAMEWORK



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ACTIVITY:

- NETWORKING - Identification of the official national partners involved in plant cryopreservation and creation of a network of national cryobanks.
- NEEDS IDENTIFICATION - Survey of cryopreservation methods used in national cryobanks for specific plant species in order to evaluate the effectiveness of the methods and the possibility of their expansion.
- RECOMMENDATIONS PUBLISHED - Review of national cryopreservation strategies including selection of plant species, type of plant material, health status, minimum number of safely stored specimens, minimum plant recovery, storage facilities, staff involvement, etc. to develop a concept for European standards for plant cryopreservation.



ECPGR Cryopreservation Working Group

ACTIVITY:

February 2022

The ECPGR Working Group on Cryopreservation was established.

August 2022

Miloš Faltus (Czech Republic) and Bart Panis (Belgium) were appointed as co-chairs of the Cryopreservation Working Group.

December 2022

The project on 'Genotyping-by-sequencing of the European garlic collection to develop a sustainable ex situ conservation strategy (Garli-CCS)' – submitted by the Allium, Cryopreservation, and Documentation & Information Working Groups

May 2023

The first meeting of the Cryopreservation Working Group will be held in Prague, Czech Republic, from 3-4 May 2023

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Objectives

- Obtain information about the current status of cryopreservation and the presence of long-term storage facilities as well as of experienced cryopreservation researchers
- Ensure that plant cryopreservation research in Europe is maintained at a critical mass to enable continuing advances in the science
- Ensure that European Crop collections conserving species that produce non-orthodox seeds have access to cryopreservation technologies in order to store their germplasm safely for future generations
- Investigate the possibility for cryopreserving orthodox seed collections with a focus on species with short-lived seeds including many vegetable such as leeks, onions and parsley
- Explore the application of cryopreservation to crop wild relatives or wild species and trees
- Increase collaboration between European scientists and institutes holding crop collections to establish a European Cryo-hub
- Create one or more cryopreservation back-up facilities
- Develop new biobank technologies and data management systems for cryopreserved collections



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ACTIVITY:

A joint ECPGR project of the *Allium* WG and the Cryopreservation WG.

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ECPGR Activity Grant Scheme Proposal Form

Sixth Call – Phase X (2019-2023)

Activity Proposal

Activity	
Full title	Genotyping-by-sequencing of the European garlic collection to develop a sustainable <i>ex situ</i> conservation strategy
Acronym (or short title)	Garli-CCS (Garlic Cryopreservation & Conservation Strategy)
Duration of Activity (in months)	24 months
Start date – End date <i>Please indicate start date not earlier than 3 months after deadline of Call</i>	1 st April 2023

Applying Working Group(s)

	Working Group	Indicate name and surname of Working Group Chair
1.	Allium	Helena Stavěliková
2.	Cryopreservation	Bart Panis and Miloš Faltus
3	Documentation and Information	Stephan Weise

Activity Coordinator

Activity Coordinator	
Name and Surname	Manuela Nagel
Working Group	Allium Working Group
Nationality	German
Current position	Head of Cryo and Stress Biology Research Group
Institute	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
Country	Germany
Telephone	+49 39482 5156
Email	Nagel@ipk-gatersleben.de

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Activity Partners (ECPGR-funded)

Please note that each partner needs to be a member of an ECPGR Working Group to be eligible for funding. For self-funded partners please use the separate box below.

Partner ID No.	Name and Surname	Institute	Country
1	Manuela Nagel	IPK	Germany
2	Līga Lepse	Institute of Horticulture	Latvia
3	Helena Stavěliková	Crop Research Institute	Czech Republic
4	Terhi Suojala-Ahlfors	Natural Resources Institute Finland (Luke)	Finland
5	Priit Põldma	Estonian University of Life Sciences	Estonia
6	Florence Esnault	INRAE	France
7	Danguolė Juškevičienė	Lithuanian Research Centre for Agriculture and Forestry (LAMMC)	Lithuania
8	Jelka Šuštar Vozlič, Mojca Škof	Agricultural Institute of Slovenia	Slovenia
9	Catherine Cook	Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization-Dimitra	Greece
10	Smiljana Goreta Ban	Institute of Agriculture and Tourism	Croatia
11	Isabel Gomes da Silva	Portuguese Genebank (BPGV)	Portugal
12	Marcelino de los Mozos Pascual	Regional Institute for Research and Agrifood and Forestry Development of Castilla - La Mancha (IRIAF)	Spain
13	Vincenzo Candido	Department of European and Mediterranean Cultures - DiCEM	Italy
14	Miloš Faltus	Crop Research Institute	Czech Republic
15	Mariusz Chojnowski, Denise Fu Dostatny, Anna Wawrzyniak	National Institute for Horticultural Research (INHORT)	Poland

16	Erik de Vahl	Swedish National Genebank for Vegetatively Propagated Horticultural Crops	Sweden
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Self-funded partners

Partner No.	Name and Surname	Institute	Country
1	Stephan Weise	IPK / EURISCO	Germany
2	Domingo José Rios Mesa, Desirée Afonso Morales	Center for Conservation of Agrobiodiversity of Tenerife (CCBAT)	Spain
3	María Victoria Colombo Rodríguez and Isabel Fernández Navarro	Madrid Institute for Rural, Agricultural and Food Research and Development (IMIDRA)	Spain
4	María Cristina Alcántara Rodríguez	Regional Institute for Research and Agrifood and Forestry Development of Castilla - La Mancha (IRIAF)	Spain
5	Maarten van Zonneveld	World Vegetable Center	International

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4. Cryopreservation training (October 2023 - December 2024)

WP 4.1 Partner 14 representing the CRYOPRESERVATION group will organize for the consortium members, a cryopreservation workshop in Prague. Overall, 7 participants and 2 experts from abroad will be invited to discuss procedures, provide guidance and training on garlic cryopreservation. Other partners can join the lectures online.

WP 4.2. All partners implementing cryopreservation will be involved to develop a **cryopreservation strategy** which includes thresholds for the number of shoot tips for safety duplicates, the number of replicates, locations for safety duplications and additional support by online training and meetings. The cryopreservation strategy should be considered a model for clonal plants. Therefore, a two-day virtual meeting is planned. The first day of the meeting will summarize the experiences of the workshop organizers and participants. The second day will be devoted to a discussion of the cryopreservation strategy. The number of participants will not be limited. This meeting will take place at the end of 2024.

5. Garlic conservation strategy (Whole project period)

WP 5. All partners will discuss a conservation strategy in **four online meetings** taking place in the 1., 6-7th., 12-14th and 18-20th project months and **one final face-to-face meeting at IPK in 22-24th months**. GBS data available from IPK collection will be used as the preliminary dataset for discussion of the following topics

- Determinants and thresholds for unique accessions
- Determinants and thresholds for duplicate accessions
- Prioritization of garlic accessions for cryopreservation
- Prioritization of garlic accessions for a European Core Collection
- Gap analysis and acquisition strategy

Table 1. On basis of the work carried out in WP1 to WP5, we schedule the work as follows:

	1 st y	2 nd y
WP 1.1 Sampling	█	
WP 1.2 Sequencing	█	
WP 1.3 GBS analysis		█
WP 2.1 Phenotyping		█
WP 2.2 GWAS		█
WP 3.1 Data upload		█
WP 3.2 Links to EURISCO		█
WP 4.1 Cryopreservation training		█
WP 4.2 Cryopreservation strategy		█
WP 5 Development of Garlic conservation strategy		█
Meetings	Online	Online
Publication		Online
		Physical

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Expected products and related ECPGR Objectives

List concrete products and results that are obtained by the Activity and the corresponding number(s) of the ECPGR Outcome(s) and/or Output(s) and/or Activities to which each product/result will contribute.

	Expected products/results	Corresponding ECPGR output, activity
1	Publicly available sequencing data of 1,800 garlic accessions	To promote use of PGRFA
2	Additional phenotypic data for ability to produce scape, bulb structure type and shape of mature bulb of 1,800 accessions	To provide passport and phenotypic information through the EURISCO catalogue
3	Identification of unique and duplicated accessions	To efficiently conserve and provide access to unique germplasm
4	GWAS analysis	To promote use of PGRFA
5	Cryopreservation training	To efficiently conserve and provide access to unique germplasm
6	Development of a cryopreservation strategy	To efficiently conserve and provide access to unique germplasm
7	Development of a garlic conservation strategy	To efficiently maintain and provide access to unique germplasm

Workplan for the proposed period of the Activity

Brief description of meetings and/or main actions of the Activity.

	Type of Action (indicate if "meeting" or "other action")
1	First Online meetings (1. Month) <ul style="list-style-type: none"> ● Agreement about the planned schedule ● Agreement about delivered material, protocol exchange to collect leaf material and to conduct phenotyping
	Second Online meeting (6-7 th month) <ul style="list-style-type: none"> ● Preliminary data are used to discuss about threshold levels for number of unique alleles and duplicates ● Identification of unique accessions and duplicates
	Third Online meeting (12-14 th month) <ul style="list-style-type: none"> ● First sequencing can be used for decision making processes ● Cryopreservation group will be involved to talk about first concepts
	Fourth Online meeting (22-24 th month) <ul style="list-style-type: none"> ● Data publication, GWAS, and manual development ● Cryopreservation group will be involved to discuss cryopreservation strategy
2	Sequencing (2 nd – 9 th month)
3	Phenotyping (April – December 2023 and April – September 2024)
4	Data upload (December 2023 and 2024)
5	Cryopreservation training (October - December 2024)
6	Meeting: Final discussion about cryopreservation and conservation strategy (April 2025)

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1		
2		
3	NETWORKING	NEEDS IDENTIFICATION
4	GWAS analysis	To promote use of GRFA
5	Cryopreservation training	To efficiently conserve and provide access to unique germplasm
6	Development of a cryopreservation strategy	To efficiently conserve and provide access to unique germplasm
7	Development of a garlic conservation strategy	To efficiently maintain and provide access to unique germplasm

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	Type of Action (indicate if "meeting" or "other action")
	First Online meetings (1. Month)
	<p style="text-align: center;">ACCESSIONS CRYOPRESERVED</p> <p style="text-align: center;">CRYOPRESERVATION FRAMEWORK</p> <p style="text-align: center;">RECOMMENDATIONS PUBLISHED</p>
	<ul style="list-style-type: none"> • Cryopreservation group will be involved to talk about first concepts
	<p>Fourth Online meeting (22-24th month)</p> <ul style="list-style-type: none"> • Data publication, GWAS, and manual development • Cryopreservation group will be involved to discuss cryopreservation strategy
2	Sequencing (2 nd – 9 th month)
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ECPGR Cryopreservation Working Group

ECPGR Cryopreservation WG members:

18 countries

33 members

1st ECPGR Cryopreservation WG meeting:

14 countries

21 members + 1 observer

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13 countries

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ECPGR Cryopreservation Working Group

European institutes holding cryopreserved collections (according the feasibility study of 2017)

- Alliance of Bioversity International and CIAT, **Belgium**
- CRI (Crop Research Institute), **Czech Republic**
- LUKE (Natural Resources Institute), **Finland**
- IRD (Institute of Research for Development), **France**
- JKI (Julius Kühn-Institut), **Germany**
- IPK (Leibniz Institute of Plant Genetics and Crop Plant Research), **Germany**
- CNR (National Research Council), **Italy**
- RIH (Research Institute of Horticulture), **Poland**

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ECPGR Cryopreservation Working Group

Meeting members

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- Institute for Plant Genetic Resources, **Bulgaria**
- CRI (Crop Research Institute), **Czech Republic**
- LUKE (Natural Resources Institute), **Finland**
- INRAE National Research Institute for Agriculture, Food and Environment, **France**
- LEPL Scientific Research Center of Agriculture, **Georgia**
- JKI (Julius Kühn-Institut), **Germany**
- Council for Research in Agriculture and Economics, **Italy**
- CNR (National Research Council), **Italy**
- Plant Breeding and Acclimatization Institute – National Research Institute, **Poland**
- INIAV Instituto Nacional de Investigação Agrária e Veterinária, **Portugal**
- Fruit Research Institute, **Serbia**
- University of Maribor, **Slovenia**
- CITA Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA), **Spain**
- Universidad Politécnica de Madrid, **Spain**
- CSIC Agrobiological Research Institute, **Spain**
- Aegean Agricultural Research Institute, **Türkiye**

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Goals:

- *Overview of the current state of plant cryoconservation in Europe (May 3rd)*
- *Discussion about the concept for a rational and efficient way to collaboratively conserve the relevant European plant genetic resources through cryopreservation (May 4th)*



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NETWORKING - other European institutes/countries to be involved?

EXPERIENCE SHARING - Need for training? If so, on what topics?

- *Funding possibilities for collaboration?*

STRATEGY FOR CRYOCONSERVATION

- *Priority crops?*
- *Other applications of cryopreservation - Virus eradication - Conservation of materials with specific characteristics (totipotent cell lines, lines with specific metabolites, elite clonal breeding lines or genetic stocks) - Cryopreservation of orthodox, intermediate and/or recalcitrant seeds?*
- *Need for a Cryopreservation back up (cfr Svalbard seed vault)?*

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- sharing experience
- skills training
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**Thank you for your
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