European Cooperative Programme for Plant Genetic Resources
ECP/GR

Report of a Working Group on Cryopreservation

First Meeting, 3-4 May 2023, Prague, Czech Republic

M. Faltus, B. Panis and L. Maggioni

The European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative programme among most European countries aimed at contributing to rationally and effectively conserve *ex situ* and *in situ* plant genetic resources for food and agriculture, provide access and increase utilization (http://www.ecpgr.org). The Programme, which is entirely financed by the member countries, is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries. The Coordinating Secretariat is hosted by The Alliance of Bioversity International and CIAT. The Programme operates through Working Groups composed of pools of experts nominated by the National Coordinators. The ECPGR Working Groups deal with either crops or general themes related to plant genetic resources (documentation and information, *in situ* and on-farm conservation and cryopreservation). Members of the Working Groups carry out activities based on specific ECPGR objectives, using ECPGR funds and/or their own resources.

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Cryopreservation lab of the Federal Ex Situ Genebank for agricultural and horticultural plants at the Leibniz Institute (IPK). Courtesy of Leibniz Institut (IPK)

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SUMMARY REPORT OF THE MEETING

Introduction

The first meeting of the Working Group (WG) on Cryopreservation of the European Cooperative Programme for Plant Genetic Resources (ECPGR) was held on 3-4 May 2023 in Prague, Czech Republic. It was organized in collaboration with the Crop Research Institute.

Upon consideration that cryopreservation is a safe, long-term complementary conservation method, with low maintenance cost, suitable for recalcitrant seeds and clonal crops, and that expertise in Europe exists but there is limited coordination of efforts, a proposal to establish a Working Group on Cryopreservation was submitted to the Secretariat in October 2021 by Nicolas Roux and Bart Panis, Alliance of Bioversity International and CIAT, together with Manuela Nagel, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany and Stephane Dussert, Institute of Research for Development (IRD), Montpellier, France (see letter of submission in Appendix I).

The proposal was endorsed by the ECPGR Steering Committee (SC) in February 2022. At its 16th meeting in June 2022, the SC suggested nominating two co-Chairs, with the understanding that they should guide the WG according to the interests of the European region. According to the expectations of the SC, the WG should develop a concept for the most rational and efficient way to collaboratively conserve under cryopreservation the relevant European material. A suitable number of European facilities that could share the tasks could be defined. Genebanks that are currently working on cryopreservation should be able to share and further develop their knowledge, also expanding it to other crops or genebanks, as appropriate, to collectively serve the regional needs.

Based on received 'Expressions of Interest', the ECPGR Executive Committee (ExCo) nominated Miloš Faltus (Czech Republic) and Bart Panis (Belgium) as co-Chairs of the Cryopreservation WG in August 2022. A request to hold the first meeting was also accepted by the ExCo, with the main goal of the event being to get to know each other within the ECPGR WG and establish cooperation between European experts on cryopreservation, provide and share information on cryopreservation activities in European countries, discuss the main elements to be considered for the elaboration of a cryopreservation strategy in Europe and a platform for sharing information for a greater expansion of cryopreservation methods, increasing their reliability and routine use.

Opening of the meeting

Milos Faltus, co-Chair of the WG, and Mikulas Madaras, Director of the Crop Research Institute (CRI), welcomed the participants from 12 countries.

M. Madaras briefly introduced CRI, which is the centralized seat of the Czech seed and cryobanks. Cryopreservation is established as a safety-duplicate approach for field and *in vitro* collections of vegetatively propagated crops. The vitrification method is used according to optimized conditions of cryo protocols. The cryobank closely cooperates with international partners, such as with the established trilateral agreement with Germany and Poland for garlic cryopreservation. A bilateral Czech-Ukrainian garlic cryobank is also being prepared.

Vojtech Holubec, Czech National Coordinator, also welcomed the participants on behalf of the national programme for plant genetic resources conservation and utilization, of which the cryopreservation group is a key member. He also reminded that the Czech group was the first

to propose the establishment of a collaboration on cryo activities within ECPGR about 15 years ago, under the leadership of Jiří Zámečník.
The co-Chairs Milos Faltus and Bart Panis alternated and supported each other in chairing the entire meeting.

Information on ECPGR

Lorenzo Maggioni, ECPGR Secretariat, Rome, Italy

The objectives for ECPGR Phase X (2019–2023), the membership, funding, structure and mode of operation of the programme were presented, indicating that the Cryopreservation WG had become the 24th WG of the programme. The main pillar initiatives of ECPGR were briefly mentioned: 'A European Genebank Integrated System' (AEGIS), with the objective to conserve, in a collaborative way and at agreed quality standards, the genetically unique and important accessions for Europe of all crops, and to make them available for breeding and research through the Standard Material Transfer Agreement (SMTA); EURISCO (the European Search Catalogue for Plant Genetic Resources), maintained and developed by IPK, Gatersleben, Germany, on behalf of ECPGR; EVA (European Evaluation Network), a coordinated framework to promote private–public partnership among breeding companies and public genebanks or research institutes.

Involvement of the ECPGR Secretariat in EC-funded projects had recently contributed to the publication of a *Plant Genetic Resources Strategy for Europe*, which is encouraging the establishment of a cryopreservation network to improve management strategies and methods for clonally-propagated plants (e.g. fruit trees) and recalcitrant seeded plants.

An introduction to plant cryopreservation

Bart Panis, Katholieke Universiteit, Leuven, Belgium

B. Panis, co-Chair of the WG, based at the University of Leuven, which is hosting the Belgium office of the Alliance of Bioversity International and CIAT, was happy to see a continuation of efforts made in Europe on cryopreservation research. He remembered the start of collaboration 20 years ago with the EC-funded project CRYMCEPT on fundamental aspects of cryopreservation, followed by a specific COST action (COST Action 871 'CryoPlanet', Cryopreservation of crop species in Europe) and now the ECPGR WG.

He introduced the concept of cryopreservation, a process in which cells or whole tissues are preserved by cooling to low sub-zero temperatures, typically around −196 °C. At this temperature, any biological activity ceases. The main challenge is to avoid freezing injuries. It is therefore important to maintain water in a state that is called vitrification, which avoids the creation of ice crystals. This can be achieved through ultrarapid freezing, which is fast enough to prevent water molecules from organizing into ice, and with the use of compounds interfering with the organization of ice crystals. Different cryopreservation methodologies were summarized, indicating that the most used are dormant buds cryopreservation (no *in vitro* steps are required, but applies only to bud-forming and grafted crops) and vitrification through the preparation of suitable solutions, in particular 'droplet vitrification', which is very successful ultrafast vitrification. The main applications of the methodology were considered: 1) storage of genetic resources, 2) long-term storage of specific cell lines, 3) eradication of viruses, 4) breeding tool, and 5) storage of clean stock cultures.

A survey on the global status of cryopreservation was carried out in 2017, showing that 15 institutes together hold ca. 10,000 accessions of 30 crops in cryopreservation and that only 17 crops have cryopreserved collections of more than 100 accessions. Europe plays an important role since the majority of accessions is cryopreserved by European institutes (such as banana in Belgium, elm in France, strawberry and potato in Germany, garlic in the Czech Republic and in Germany, and citrus in Italy). A challenge for the future of plant cryopreservation is the extension of efficient protocols to some species that are still recalcitrant to the existing protocols. Also, mere survival in some cases needs to be improved into efficient growth. Cryopreservation remains a labour-intensive and thus costly activity which should be seen as a long-term investment, considering that only 50-70 accessions can be introduced into cryo per skilled staff per year. The investment pays off in the long-term considering that material will be safely preserved for at least 20-30 years.

ECPGR Cryopreservation Working Group

Milos Faltus, Crop Research Institute, Prague, Czech Republic

The rationale for increasing cryopreservation of plant genetic resources is well understood. However, cryopreservation is not yet a standard operation in this field, due to the need to acquire specific knowledge and know-how, the difficulty to implement the methodology in different labs and the differential response of various genotypes. These limitations can be overcome by sharing experiences, providing training and setting standards. The WG was established for this purpose.

One of ECPGR's objectives is the increased efficiency of plant germplasm conservation, and cryopreservation is an essential development in this direction. The need for the establishment of the WG is shown by ECPGR's recommendations to work towards a regional cryopreservation framework. Indicators of success will ultimately be an increase of cryopreserved accessions. The activities of the WG include networking, identification of needs and setting standards. Since its establishment in February 2022, two co-Chairs were appointed in August 2022 and the WG was involved in an ECPGR Grant Scheme-funded 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. Garlic is here used as a model crop with the intention to define a European cryopreservation strategy, which may be extended to other crops in the future.

The objectives of the Cryopreservation WG are defined in the establishment document (see full document in Appendix I) and include:

- Gather information on the status of cryopreservation in Europe
- Promote plant cryopreservation research in Europe
- Provide access to cryopreservation technologies for European non-orthodox seed collections
- Extend cryopreservation to orthodox seed collections of short-lived seed crops
- Explore the application of cryopreservation to crop wild relatives
- Increase collaboration between European scientists and institutes holding crop collections to establish European Cryo-hub(s)
- Create one or more cryopreservation back-up facilities
- Develop new biobank technologies and data management systems for cryopreserved collections.

Currently, the WG is counting 33 members from 18 countries. This meeting was attended by 21 members and 1 observer from 14 countries, which is a very good basis for extended collaboration. The goals of this meeting were to obtain an overview of the current status of plant cryopreservation in Europe and to discuss about a rational and efficient way to collaboratively conserve the relevant European plant genetic resources through cryopreservation, as well as provide opportunities to network, share experiences, organize training and set standards.

What is inside your cryobank?

David Ellis, International Potato Center (CIP)

D. Ellis, formerly responsible for USDA and CIP collections, a former consultant for the Crop Trust in respect of cryopreservation activities, and currently a consultant for CGIAR, was invited to share his experience with the WG.

Connected remotely, D. Ellis described the evolution of cryobanking with plants. While before the 1980s everything surviving was considered a success, the current target is to obtain plants that can grow well outside of test tubes. Unfortunately, no uniform standards or guidelines are followed, many genebank managers are averse to throwing anything away and thus most cryobanks are storing a lot of material that is of no use. It is very important to do housekeeping and clean the cryobank. In 2013-14, the CIP genebank disposed of more than 50% of the potato and more than 90% of the sweet potato cryo collection, since they were either not viable or contaminated. Often cryobanks are leftovers of research or archived material. Cryobanks should rather be reserved for material that can be of use to future generations, with written standards of excellence in place and followed, well-defined operational protocols, and well-documented in the database. Everything must be predictable for future genebank managers, ensuring high quality, verified identity and phytosanitary cleanliness.

A few points to consider in plant cryo: reports in literature cover more than 40 crops, but only a handful of them are extensively conserved in cryo; there is a critical need to secure long-term conservation of plant genetic resource (PGR) collections, but not everyone needs to actively engage in cryopreservation; many different plant tissues can be cryopreserved and there is not one single method that fits for everything; most cryo methods require *in vitro* (exceptions are pollen, seed and dormant buds). *In vitro* steps can thus be the limiting factor; genotyped and disease-free starting material is critical.

An independent expert cryo feasibility study was published in 2017 by Bioversity International[1]. It was commissioned to investigate the feasibility and need of a safety back-up facility for cryopreserved collections of vegetatively propagated and recalcitrant seed crops.

The conclusions were that cryo is the best long-term conservation option for plants that cannot be stored as botanical seed. However, there is a high initial cost. Cryo has benefits for long-term safety back-up. There is a need to accelerate the development and implementation of cryopreservation since *in vitro* and field collections remain at risk. CGIAR is ideally positioned to work out a proposal to follow up with the feasibility study.

The cost of cryopreservation is high. For example, at CIP, the cost of putting a potato

[1] Acker, J.P.; Adkins, S.; Alves, A.; Horna, D.; Toll, J. (2017) Feasibility study for a safety back-up cryopreservation facility. Independent expert report: July 2017. Rome, Italy: Bioversity International, 100 p. ISBN: 978-92-9255-073-8. https://hdl.handle.net/10568/91009

accession into cryo is ca. US$400 and once in cryo, the cost of maintaining one accession is $7 per year. Compared to *in vitro* maintenance, savings start to accrue after six years and then continue year after year. Today, 90% of the CIP potato collection is in cryo. The conversion can be done relatively quickly, but only with a concerted effort. A Global Plant Cryopreservation Initiative is envisaged, with a focus on recalcitrant and clonal crop collections in the developing world. It will focus on capacity building and establishing a global plant cryo network, with regional hubs acting as centres of excellence, based at CGIAR institutions. These will convert research results into operational protocols and provide capacity building and operational services to place genetic resources into cryo and safety back up facilities. A Global Plant Cryo Community of Practice is also envisaged and recommended, and the ECPGR WG should be part of it. A fundraising campaign is currently ongoing to convert the vision into effective implementation.

Country presentations

(Country reports are available in Appendix IV, page 27)

Belgium

Bart Panis, Katholieke Universiteit, Leuven

Since 1985, the banana world collection has been based at the Katholieke Universiteit (KU) in Leuven. In 1994, this became an established 'in trust' collection based on the FAO International Treaty on Plant Genetic Resources for Food and Agriculture. The base collection with 1,195 accessions is cryopreserved and a large part of this is safely backed-up at IRD in Montpellier, France. The active collection is maintained *in vitro* with 1,682 accessions. The Belgian location is very convenient considering the absence of any local phytosanitary risk due to the import of banana material. A germplasm health testing system is in place to avoid the transfer of virus infections. The cryopreservation system was developed in house and is based on droplet vitrification, involving the excision of meristems from *in vitro*-rooted banana plants. This step requires skilled staff with specific training.

Research was coordinated here through the EC-funded project CRYMCEPT (Establishing Cryopreservation Methods for Conserving European Plant Germplasm collections) (2002-2005). The project investigated physio-biochemical parameters related to plant cryopreservation. Another initiative was the COST Action 871: "Cryopreservation of crop species in Europe', which was a networking initiative chaired by Belgium, with 16 participating countries. As part of this action and in collaboration with the International Society for Horticultural Science (ISHS), the first International Symposium on Cryopreservation in Horticultural Species was held in Leuven in 2009. Over the past 25 years, about 140 researchers from 44 countries were trained on plant cryopreservation techniques in Leuven, funded by various donors. Other cryopreservation activities are carried out in various Belgian institutions, regarding azalea (ILVO), *Abies nordmanniana* (CRA-W), chicory (KU Leuven), *Maesia lanceolata* and *Medicago truncatula* (U Gent) and stevia (KU Leuven).

Bulgaria

Stanislava Stateva, Institute for Plant Genetic Resources, Sadovo

The Institute of Plant Genetic Resources in Sadovo is the seat of the national genebank and also hosts the Plant Biotechnology Laboratory, which has a long tradition of *in vitro* cell culture research and micropropagation. Specific expertise exists for *in vitro* propagation of potatoes, vines and mint with a high multiplication factor. Also *in vitro* methods for accelerated reproduction and rooting of essential oil, decorative, technical and medicinal plant species have been studied and optimized.

The creation of a cryobank is planned for the long-term conservation of potatoes, and medicinal and flower plants.

Czech Republic

Miloš Faltus, Stacy Hammond Hammond, Olena Bobrova, Alois Bilavčík, Jiří Zámečník, Crop Research Institute, Prague

In the Czech Republic, the National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity is organized by the Ministry of Agriculture and coordinated by the Crop Research Institute. The board of plant genetic resources is composed of curators of generatively and vegetatively propagated crops. Generatively propagated crops are stored as seeds at low temperatures for a few or tens of years in the Central Seed Genebank. Vegetatively propagated crops are stored as tubers, bulbs, cuttings, *ex vitro* explants or intact plants in field conditions and backed-up in the Central Cryobank.

National curators of vegetatively propagated crops are respectively based at the Potato Research Institute Havlíčkův Brod (potato *in vitro*), Hop Research Institute Žatec (hop), MENDELU Lednice (thermophilic temperate fruit trees), CRI Olomouc (*Allium*), VSV Karlštejn CRI, Ampelos Vrbovec, MENDELU Lednice (*Vitis*), and Research and Breeding Institute of Pomology Holovousy (temperate fruit trees).

The strategy of plant germplasm cryopreservation foresees the safety duplication of basic collections (with different storage methods and localities) and storing the most valuable genetic material of Czech origin. The central cryobank collaborates with plant germplasm curators, who provide the most valuable samples for their backups.

Currently, 500 accessions are under cryopreservation at the Central Cryobank, *Allium sativum* L. (187), *Solanum tuberosum* L. (104), *Humulus lupulus* L. (68), *Fragaria* x *ananassa* (34), *Lonicera* L. (edible) (24), *Pyrus communis* L. (24), *Malus domestica* BORK (17), *Prunus armeniaca* L. (12), *Cerasus vulgaris* P.M (10), *Malus* MILL. (6), *Persica vulgaris* P.M (5), *Cerasus avium* (L.) M (3), *Cerasus* P. MILLER (3), *Vitis vinifera* L. (3).

Funding is ensured by international (37%), national (32%) and institutional (22%) projects and by the national programme (9%).

Cryopreservation protocols are developed with the use of either *ex vitro* or *in vitro* plant material, acclimation is based on low temperatures and osmotic procedures. Methods used are two-step freezing, encapsulation-dehydration, simple dehydration, vitrification and droplet-vitrification. Standards are optimized for the safe recovery of samples after cryopreservation.

The two-step freezing involves dehydration by freezing. It is used for dormant buds. A second group of protocols is based on dehydration by dry air (encapsulation-dehydration and simple-dehydration). The last group of protocols is based on vitrification, mostly using droplet vitrification. For recovery, Stefan Dussert's probability tool is used. With a minimum of 120

stored shoot tips, 40 control samples and an explants recovery rate of at least 30%, the minimum number of recovered shoot tips from the total stored will be 14.

Cryotherapy has been applied to virus elimination by cryopreservation of potato, hop, garlic and raspberry.

Thermal analysis by differential scanning calorimetry is used to measure heat flow during temperature change and assess heat capacity changes connected with state of matter changes.

Prospects for improvement of cryopreservation activities include enhancing knowledge of cryotolerance, developing protocols for sensitive plant species and genotypes, completing the cryopreservation of selected types of crops of national importance, and ensuring control of the health status of explants. Sharing information about cryobanking and cooperating in international projects are crucial. Limitations are mainly linked to unstable and insufficient funding for the cryobank.

Finland

Anna Nukari and Saija Rantala, Luke (Natural Resources Institute), Helsinki

Cryopreservation of PGR is being executed under the national PGR programme (Finnish National Genetic Resources Programme for Agriculture, Forestry and Fishery, 2018) at the Natural Resources Institute Finland (Luke). The plant accessions chosen for long-term preservation by cryopreservation are maintained in the Luke cryobank in gas phase tanks below -150˚C. The Luke PGR network for long-term cryopreservation, research and protocol development and safety duplication includes cryopreservation locations at Haapastensyrjä, Jokioinen and Suonenjoki. Cryopreservation at Luke starts with crop prioritization, which is dedicated to vegetatively propagated horticultural plants, mainly *Rubus*, *Ribes*, *Fragaria*, *Malus*, *Prunus*, *Solanum* and some ornamental plants. On-going research is dedicated to *Allium cepa* Aggregatum group, *Humul*us, *Ribes rubrum* and *Fragaria.*

Plant materials used for cryopreservation are apical or lateral buds collected from micropropagated plants, dormant buds collected from the field or greenhouse (*Ribes*, *Prunus*, *Malus*), pollen and seeds (*Rhododendron*). Cryopreservation methods are tested and optimized, plant materials are then pretreated and frozen. Regeneration of the cryopreserved lots is checked and long-term cryopreservation is monitored by thawing control samples at fixed periods. A few projects on cryopreservation are currently funded, such as the GEHO KRYO project for cryopreservation backup of the national PGR collections, the KASVIKRYO project on the development of the PGR cryobank and the Strawberrycryo project on the cryopreservation of the pre-basic mother materials of strawberry cultivars in the certified plant production. The latter is executed at Luke, mainly at Haapastensyrjä, under the statutory services programme, as part of the certified plant production scheme, in collaboration with the National Food Authority.

Cryopreservation of strawberry pre-basic mother materials of certified production ensures enhanced safety and secured true-to-cultivar preservation of strawberry cultivar materials and reduces the expenses of repeated *in vitro* initiations and plant disease and true-to-type testing. The strawberry protocol involves the excision of apical buds from cold-pretreated *in vitro* cultures and pretreatment on sucrose media.

The expectation form the Cryopreservation WG is to bring together experts for cryoconservation of plant genetic resources around Europe to share information, discuss strategies for cryoconservation of PGR in Europe and broaden the routine use of cryopreservation methods.

France

Philippe Chatelet, INRAE, Montpellier

Cryopreservation efforts in France include seed cryobanking, vegetatively propagated species cryopreservation and cryotherapy. Presently, *Brassica* seeds of 106 populations (907 *B. oleracea*, 71 *B. napus* and 38 *B. rapa*) are conserved in liquid nitrogen as five tubes of 250 seeds each. The aim is to safety-duplicate these populations of orthodox seeds with high lipid content, requiring regeneration in 5-year cycles. In the case of coffee, intermediate seeds are cryopreserved after dehydration. Presently, 337 *Coffea* accessions from the Réunion Island collection have been cryopreserved, i.e. 62% of the accessions. Cryobanking of *Citrus* seed is under development with preliminary experiments and is planned to get started in Corsica. These intermediate seeds are highly heterozygous, but the presence of nucellar embryos in 70% of all seed-bearing accessions allows maintenance of the original genetic combination through seed cryobanking.

Cryopreservation of vegetatively propagated species is ongoing for potatoes, mainly *Solanum tuberosum* (78 clones) and 25 other related species conserved after PVS2 treatment and droplet vitrification. At present, 152 clones have 'validated' cryopreserved samples (i.e. with a regeneration rate over 40%) with 100 apices per clone. In the case of *Malus*, protocols have been improved between 2011–2015, using dormant bud technique and droplet vitrification. As of 2023, 193 *Malus* accessions have been cryopreserved. Development of a vitrification technique for pear is in progress.

Cryopreservation of embryogenic cultures is practised for *Hevea* and *Coffea*, to conserve embryogenic lines used for experimental and breeding purposes.

Activities related to cryotherapy are ongoing through projects related to *Solanum* species, *Ananas* species, Yam and *Vanilla*. Sanitation by cryotherapy of 3,000–3,500 *Vitis vinifera* accessions belonging to the French grapevine repository is planned.

In conclusion, several sizeable cryobanks have already been implemented for *Brassica*, *Coffea*, *Malus*, *Solanum* and forest species). Other cryopreservation applications are routinely used (*Hevea*, *Coffea*). The technique is being considered for backing-up vegetatively propagated species and for phyto-sanitation, although resources and facilities for a systematic approach are lacking.

Georgia

Levan Ujmajuridze, LEPL Scientific Research Center of Agriculture, Tbilisi

In Georgia, cryopreservation is used for the conservation of genetic material of animals, but there is no relevant infrastructure for plants in the country yet.

In future plans, cryopreservation will need to target some priority crops, such as grapevine (*Vitis vinifera sativa* L.), which is one of the most important crops in Georgia. More than 1,000 autochthonous grape varieties, with 140 accessions of wild vines (*Vitis vinifera sylvestris* L.) are now maintained at the Scientific Research Centre of Agriculture, Jighaura collection.

A recent research on cryopreservation of chestnut (*Castanea sativa* Mill.) was carried out in Georgia during 2019–2021. Chestnut is the dominant species of mountainous forests of Western Georgia and holds vulnerable status (VU) in the IUCN Red List of Georgia. Cryopreservation is considered the most efficient way to conserve this non-orthodox seed species. As a result, an optimized cryopreservation protocol for embryonic axes of chestnut

has been developed, based on the encapsulation-vitrification procedure.

Cryotherapy is considered to have a good potential in Georgia for virus eradication.

Collaboration within the WG will be essential for capacity building of cryopreservation in Georgia.

Germany

Status of the National cryo-collection

Manuela Nagel, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben

On behalf of M. Nagel, M. Höfer presented the status of the German cryo-collection. Overall, IPK maintains ca. 144,000 accessions as seed in cold storage, 2,650 accessions in field genebanks, 3,150 accessions in *in vitro* slow-growth storage and 2,400 accessions under cryopreservation. sSpecifically, the cryo-collections regard potato (2,017 accessions), mint *Allium* species (246) and mint (147). The longest experience has been developed for potato, which started to be conserved under cryo in 1997, with constantly improving methodologies and protocols. Currently, triplicates of 3 x 65 shoots are conserved adopting a PVS3 droplet-vitrification procedure. Further improvements will be required, considering the variable genotype-dependent regrowth rate.

Ongoing cryopreservation projects focus on garlic (ECPGR-funded Garlic-CCS for the Garlic Conservation and Cryopreservation Strategy), potato (DFG-funded PotEND – Endophyte interactions in response to complex abiotic stress during potato cryopreservation), and duckweed (Duckweed Cryopreservation, funded by Philipp-Schwartz and the Humboldt Foundation).

Proposals for collaborating activities with the ECPGR WG include projects on characterization, evaluation, genotyping with other WGs, i.e. Solanaceae, Berries and Medicinal and Aromatic Plants, to support the identification of duplicates and unique material, to prioritize cryopreservation of European accessions and to guide collection management. It would also be useful to prepare a handbook of suitable and robust cryo-protocols and organize cryopreservation workshops and COST Actions. The exchange of cryo-material for safety duplication should be considered, as well as joint studies to work on fundamental mechanisms.

Preservation of fruit genetic resources in Germany

Monika Höfer, Julius Kühn-Institut, Federal research Centre for Cultivated Plants, Dresden

The German Fruit Collection is a decentralized genebank network dedicated to cultivars of fruit genetic resources, which is centrally coordinated by the Institute for Breeding Research on Fruit Crops, Dresden-Pillnitz. The aim is to ensure an effective and long-term conservation and that the genetic resources are made available for research, breeding and landscaping. Each cultivar should be preserved at least in duplicates for safety. On the other hand, accessions of wild fruit species are not part of the German Fruit Collection but are conserved as an active collection only at the Julius Kühn-Institut in Dresden-Pillnitz. In this case, wild *Malus* and *Fragaria* collections are among the largest collections in Europe, with 518 *Malus* accessions belonging to 26 primary species and 20 hybrid species. Duplication of the whole collection at a second field site or *in vitro* are not realistic solutions especially for *Malus*. A seed collection would not represent the true genotypes of clonal, heterozygous accessions. Additionally, growing genotypes from seed takes too long. Cryopreservation is considered the best option

for managing the duplicate collection as a space and cost-efficient method compared to a second field collection.

Cryopreservation of *Malus* with dormant buds was started, since this does not require *in vitro* steps and trees can be recovered in a short time. All the steps have been adapted to Central European weather and laboratory conditions, including cold pre-treatment of scions, dehydration and pre-freezing, transfer into liquid nitrogen, and then re-warming, grafting into rootstocks and recovery. The majority of accessions tested (64%) reached the 40% survival rate that is considered successful. However, the rate of response is highly dependent on genotype. Following the success with *Malus*, tests will continue with *Prunus* and *Pyrus.*

The *Fragaria* collection includes more than 200 cultivars and 286 wild species accessions belonging to 22 species. Plants are conserved in pots in the field and transferred into a greenhouse in winter. After various tests, cryopreservation of *Fragaria* is now taking place with PVS2 vitrification with pre-culture and selective dehydration steps and rehydration after cryostorage. The rate of recovery here was successful (> 40%) for all tested genotypes, with an average of 86% recovery. The protocols are now operational and already 239 accessions have been stored in cryo tanks.

In summary, the German Fruit Genebank conserves cultivars of apple, pear, strawberry, cherry and plum in the field, and strawberries under cryopreservation. The wild species collection conserves *Malus*, *Fragaria*, *Prunus* and *Pyrus* in the field and has started to conserve everything in cryo, with *Prunus* as the next species to be tested.

During the discussion, it was clarified that the testing procedure for *Malus* dormant buds cryopreservation is making use of 50 buds, of which 30 are cryopreserved and 20 are tested for recovery. It was also clarified that the rate of success depends on the genotype and not on the weather conditions during winter. It was also proposed to set up an experiment whereby the same cultivars grown in different European climatic conditions could be tested for cryopreservation in one location with the same methodology, to evaluate how different winter climate conditions affect the rate of recovery.

Italy

Tissue culture-based conservation of fruit germplasm at CREA-OFA, Rome – Italy

Emilia Caboni, CREA-OFA, Rome

The Council for Agricultural Research and Economics (CREA) maintains in Rome the *ex situ* field repository of fruit germplasm, financed by the Ministry of Agriculture, Food Sovereignty and Forests (MASAF), with 5,000 fruit species accessions (peach, apricot, plum, cherry, hazelnut, walnut, etc.). The same institute is dedicated to conserving the collection by slow growth *in vitro* culture. Ongoing research is dedicated to defining the best protocols for *in vitro* culture. Currently, 78 accessions of different fruit crops are conserved at the *in vitro* laboratory of CREA-OFA in Rome. Research to define suitable protocols for cryoconservation started in the 1990s with encapsulation-dehydration of apical tips or axillary buds excised from *in vitro* growing shoots with various degrees of maximum regrowth rates (wild pear 60%, peach 46%, mulberry 62% and hazelnut 40%). Later, studies were carried out on protocols for cryopreservation by droplet vitrification with apple, raspberry and hazelnut, with highest regrowth rates of 47%, 18% and 57%, respectively. Studies to develop protocols for Italian varieties of plum (*Prunus domestica* L.) are in progress. Plans are also underway to start stable cryopreservation for apple, plum and hazelnut varieties. These will depend on the

identification of dedicated funding sources.

The activity of preservation of woody plant genetic resources at the CNR-IBE

Maurizio Lambardi, National Research Council of Italy, Institute of Bioeconomy (CNR-IBE), Sesto Fiorentino (Firenze)

Activities of conservation of fruit trees are traditionally carried out at the Institute of BioEconomy of Sesto Fiorentino (Firenze), belonging to the National Research Council (CNR) of Italy, through field clonal collections and relatively recently also with a cryobank, established in 2017, which is growing year by year.

The field collection is based at the Experimental Station 'Santa Paolina' in Follonica (Grosseto), with 60ha dedicated to several fruit crops, especially to olive, of which over 1,025 accessions are conserved, as well as peach, pear and others. The Experimental Station activities include all aspects of PGR documentation, conservation, characterization and use, with a particular focus on micropropagation, *in vitro* conservation and cryopreservation. The cryobank is based in Sesto Fiorentino, near Florence, currently conserving 19 ancient apple varieties and 8 plum accessions with the dormant bud technique, and 24 ancient citrus accessions preserved as polyembrionic seeds. Similarly to the procedure adopted in Germany, the dormant bud technique is applicable also to Mediterranean countries, as long as the buds are pre-treated with a cold hardening treatment of two months at -5°C. It is to be noted that post-conservation pre-grafting viability tests are applied to the buds to increase the efficiency of the protocol. Apple conservation is particularly important in Italy, which is the second producer in Europe, but with only three cultivars covering 70% of production, as opposed to hundreds of varieties used in the early 20th century. The cryopreserved ancient citrus germplasm is a duplicate of the unique Medicean collection of the 'Villa Reale Medicea di Castello' in Florence. This methodology takes advantage of the presence of nucellar embryos in the citrus seed, which preserves the original genotypes.

A few ongoing projects should be mentioned:

- Development of cryopreservation of hazelnut, in collaboration with Ferrero Trad. Lux. company and Battistini Vivai, Cesena; development of dormant bud protocols in collaboration with CAV-Center for Nursery Activity, Faenza. It is remarkable that after two months of cold hardening at -5°C, the dormant bud technique can also be applied to plants overwintering in a screenhouse, where temperatures never drop below 0°C. This protocol is very effective with apple, plum and cherry, but not with pear and peach.
- Conservation of *Abies nebrodensis*, in collaboration with the Madonie Park, Sicily, as part of a LIFE4FIR project. This species is on the verge of extinction with only 30 trees remaining. A seed bank and a cryobank have been established at the Museum of *Abies nebrodensis* in Polizzi Generosa. Excised embryos, pollen and embryogenic callus from the remaining trees are being deposited in cryo in 2024.

Tissue culture-based conservation of hazelnut germplasm at CNR-IBE, Florence - Italy

Doaa Elazab, National Research Council of Italy, Institute of Bioeconomy (CNR-IBE), Sesto Fiorentino, Italy and Assiut University, Egypt

D. Elazab was involved in a research project sponsored by the company Ferrero, aiming to develop and optimize micropropagation protocols for *Corylus colurna* for the commercial production of clonal rootstocks and to develop strategies for the medium- and long-term conservation of *Corylus* spp. germplasm. In this context, activities are also directed to develop an effective procedure of cryopreservation of *C. colurna* by the 'droplet method' and 'C- and D- cryoplate', with a collaboration between CNR-IBE (M. Lambardi) and the Catholic University of Leuven (B. Panis). Cryopreservation techniques will be developed to improve the conservation of *Corylus* spp. The acquired expertise can also be applied in Egypt, addressing the preservation of endangered species like *Zizyphus spina cristi*.

Poland

Karolina Tomiczak, National Centre for Plant Genetic Resources: Polish Genebank (NCPGR), Plant Breeding and Acclimatization Institute - National Research Institute Radzików, Błonie,

Poland has four cryobanks. The Kostrzyca Forest Genebank (Kostrzyca FGB), the Polish Academy of Sciences Botanical Garden – Centre for Biological Diversity Conservation in Powsin (PAS BG-CBDC), the European Tripartite Garlic Cryobank in the Institute of Horticulture – National Research Institute in Skierniewice (InHort), and the Młochów Research Center of the Plant Breeding and Acclimatization Institute – National Research Institute (PBAI-NRI Młochów RC). The first two are mainly specialized in the long-term storage of seeds of rare and endangered plant species. In particular, Kostrzyca FGB was established in 1995 for routine cryostorage of forest plant species. One thousand sixty-four (1,064) accessions of forest trees and shrubs and 372 accessions of 237 rare and endangered herbaceous species growing in Polish forests and meadows are conserved as seeds with direct freezing. Cryopreservation is applied to dormant buds of 47 accessions of common ash (*Fraxinus excelsior* L.) and 115 accessions of two (*Quercus robur* L. and *Q. petraea* (Matt.) Liebl.) in the form of plumules (apical meristems of the embryonic axes) with vitrification. PAS BG-CBDC is the first wild flora seed cryobank in Europe, established in 1992, focusing mainly on the collection and storage of seeds of the most endangered plant species in Poland. It also maintains a cryogenic collection of embryogenic cell suspensions of *Gentiana* and independent-living fern gametophytes, through the encapsulation-dehydration method. A collection of winter dormant buds of 289 historical apple tree varieties (*Malus domestica* Borkh.) is also in progress, with at least 50 buds per variety, extending with 15–20 varieties each year.

The European Tripartite Garlic Cryobank at InHort was founded in 2011 under the framework of the EURALLIVEG project to establish a European-integrated *Allium* L. core collection, to preserve the national collections of Germany, the Czech Republic, Poland, Italy, France, and the Nordic countries. The Cryobanks Network was organized by three project partners: InHort-Poland, Crop Plant Research Gatersleben (IPK) in Germany and the Crop Research Institute (CRI) in the Czech Republic. Shoot tips isolated from bulbils (bolting forms) or cloves (non-bolting forms) of 228 garlic accessions from three partner countries are conserved with 100 explants per accession. Each year, the collection is expanded by another 10 accessions via vitrification in PVS2 and PVS3.

The cryogenic collection of potato (*Solanum tuberosum* L.) accessions at PBAI-Młochów RC

was initiated in 2005 as part of the National Centre for Plant Genetic Resources. Shoot tips of 56 outstanding diploid interspecific hybrids are conserved via vitrification in PVS2. Pollen grains of 96 diploid hybrids, 19 diploid wild *Solanum* L. species, and 17 tetraploid *Solanum tuberosum* L., including varieties and breeding lines are conserved with direct freezing.

Despite the development of cryopreservation techniques and protocols for many plant species in Poland, a central cryobank is missing. As a result, the collections of clonally propagated plant species (except garlic and apple tree varieties) lack adequate backup, mainly due to insufficient investments in technician and specialized staff.

Provided financial support from the Ministry of Education and Science (INWEST) and the Ministry of Agriculture and Rural Development (from the Recovery and Resilience Facility) will be guaranteed, future plans aim at establishing the National Cryogenic Collection in the National Centre for Plant Genetic Resources: Polish Genebank. This plan should cover vegetatively propagated crops (potatoes, hops, fruit trees and shrubs), seeds of crop wild relatives, pollen grains of crop plant species and DNA and tissues of crop plant species (in the frame of the Global Genome Biodiversity Network).

Portugal

Octavio Serra, Intituto Nacional de Investigação Agrária e Veterinária (INIAV), Banco Português de Germoplasma Vegetal (BPVG), Braga

O. Serra described the Portuguese plant genebank, its history and collection, including nearly 45,000 accessions of several crops, with especially large collections of beans, wheat and maize. *Ex situ* conservation is organized by cold storage (active at 0–5˚C and base at -18˚C), field collection and *in vitro* storage. Field collections are dedicated to garlic, hops and medicinal/aromatic plants. *Allium* (286 accessions), *Pyrus communis* (105) and *Malus domestica* (1) are conserved *in vitro*. Many field collections exist in Portugal and the challenge will be to create safety-duplication of the unique material through cryopreservation.

Cryopreservation activities currently do not receive dedicated funding. However, priority crops for cryopreservation have been defined: garlic (activity ongoing since 2021), hop, pear and other fruit trees. The vitrification method with PVS3 is applied to garlic cloves and the cryo protocol is well established, including the viability testing. A cryo collection was started with 10 accessions as immature inflorescence stored with droplet vitrification and 30 accessions as shoot tips using the vitrification protocol.

BPGV is the only institution in Portugal performing plant cryopreservation. Main limitations are the lack of staff for routine tasks and of equipment (e.g. stereomicroscopes, horizontal flow chamber, growth chamber). Training is especially needed and there is a good expectation that this WG will be able to provide knowledge sharing.

Serbia

Tatjana Vujović, Darko Jevremović, Fruit Research Institute, Čačak

The Fruit Research Institute in Čačak is an independent research and development institute located in a renowned fruit production area of Serbia. Among its activities, breeding is carried out together with collection, characterization and evaluation of genetic resources, especially autochthonous genotypes of pome, stone, kernel and small fruit species. The *ex situ* collection comprises more than 800 genotypes of different fruit species. There is interest to reestablish here the fruit genebank of Serbia introducing, among others, different *in vitro* cryopreservation techniques. The tissue culture laboratory of the Department of Fruit Physiology undertakes

micropropagation with well-established protocols. Cryopreservation activities have started through COST actions, national or bilateral projects, aiming to optimize vitrification protocols, especially for temperate fruit species. Results of vitrification, droplet vitrification, V cryo-plate and D cryo-plate and regrowth experiments with PVS2, PVS A3 (90% PVS2 solution) and PVS3 applied to various plum, cherry, apple, blackberry, blueberry and strawberry genotypes were presented. Research on cryotherapy and pollen cryopreservation in *Prunus* spp. were also carried out.

So far, it has not been possible to establish a cryobank of temperate fruit species in Serbia due to funding limited to research projects only. The Fruit Research Institute will launch an initiative for the foundation of a national cryobank for fruit species. The establishment of the bank should be under the authority of the Ministry of Agriculture, Forestry and Water Management of Serbia. Ideally, a centralized European cryobank for fruit species could be established through a European collaborative initiative. Each partner might develop suitable protocols for their most important autochthonous genotypes and deposit specimens into the central bank.

Slovenia

Jana Ambrožič-Dolinšek, University of Maribor

At the Department of Biology, University of Maribor, an initiative is ongoing to cryopreserve a Slovenian endemic plant, *Hladnikia pastinacifolia*. It is a monotypic endemic genus, with a unique position within the Apiaceae family, with low genetic variability, an extremely narrow distribution area of 4km^2 in Trnovski gozd. It is a tertiary relic protected by legislation. Seeds are conserved *ex situ*, but are short-lived with deep dormancy, requiring several months of cold treatment. It has been important to introduce other conservation procedures, such as micropropagation (from seeds and shoots) and cryopreservation. Encapsulation-dehydration and encapsulation-vitrification were introduced. Further research will focus on droplet vitrification.

There are no national cryopreservation facilities for long-term storage in Slovenia. Therefore, it will be important to collaborate with the WG to jointly elaborate the best strategies and solutions.

Spain

M. Elena González Benito, Universidad Politécnica de Madrid, Conchi Sánchez Fernández Misión Biológica de Galicia (MBG)-CSIC Santiago de Compostela and Maria Teresa Espiau Ramirez, Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA)

The expertise of the three Spanish representatives in the WG relates to epigenetic stability analysis after cryopreservation (shoot tips, vitrification and encapsulation-dehydration) (M. E. Gonzalez Benito), cryopreservation by vitrification (shoot apex and somatic embryos) and genetic stability analysis (C. Sanchez Fernandez), and curation of the fruit tree germplasm bank, using *in vitro* conservation and aiming to use cryopreservation for long term storage (M. Espiau Ramírez).

Small cryopreservation collections exist in Spain, such as for cork oak and chestnut at the private company TRAGSA in Galicia and at MBG, Galicia. However, future plans foresee the establishment of a Spanish cryobank, starting with the crops that are currently conserved *in vitro* as shoot cultures under low temperatures, such as chestnut, oak, fruit and ornamental

trees at MBG and CULTIGAR, Galicia, and pear at CITA, Aragón.

Several collections of plant genetic resources are distributed across the country in Spain, under the coordination of the Centro Nacional de Recursos Fitogenéticos in Madrid. Vegetatively propagated species are conserved in field genebanks, in some cases duplicated in different sites. It will be necessary to back up these field collections. Only a few *Solanum* and *Pyrus* accessions are conserved *in vitro* at NEIKER (Basque country) and CITA de Aragón. Research on cryobiology is ongoing at the Universities of Valecia and Málaga and is also promoted by the Spanish Society of Cryobiology. Therefore, there is expertise in Spain, but the technology is not applied according to a national strategy for vegetatively propagated material, which should be established, provided appropriate funding for implementation can be allocated.

Türkiye

Erdinç OGUR, Aegean Agricultural Research Institute Department of Biodiversity and Genetic Resources Menemen - Izmir

On behalf of E. Ogur, Aysun Orcun remotely presented the cryopreservation activities in Türkiye. This country holds a very rich diversity of genetic resources and gives importance to its preservation for the sustainability of plant production. Conservation strategies include *ex situ*, *in situ* and biotechnological methods.

With the goal of backing up all genetic resources in a cryobank, priority for cryopreservation is given to plants with germination problems, recalcitrant seeds, plants that are difficult to preserve vegetatively and endemic or threatened plant species.

The appropriate infrastructure including liquid nitrogen tanks, sterile cabinets, material preservation tanks and other equipment (cryotubes, gloves, goggles, liquid nitrogen refill pump, and glass materials, chemicals) have been acquired, so the next step should be the establishment of a national cryobank. The species under study are *Mentha* x *piperita* L., *Allium sativum* L., *Thymus cilicius* Boiss. & Bal., *Origanum sipyleum* L., *Salvia smyrnaea* Boiss., *Prunus cerasifera* Ehrh, *Sideritis tmolea* P.H.Davis and *Galanthus elwesii* Hook f.

Details were presented about the steps of sterilization and transfer to culture medium. Protocols used for vitrification, droplet-vitrification and encapsulation-vitrification were also described, as well as the process of regeneration of the thawed samples, rooting and acclimatization to external conditions. The rate of success ranges between 50% with droplet vitrification of a local mint variety, and 80% with a different mint variety and with *Origanum sipyleum.* Garlic and *Thymus cilicius* have high success rates of 78.5% and 72.5% respectively.

The *in vitro* protocols for *Prunus cerasifera* and *Salvia smyrnaea* were established, and studies on developing cryopreservation protocols have been initiated. Studies have been started on *in vitro* propagation protocols of *Sideritis tmolea* and *Galanthus elwesii*.

Limitations to the expansion of cryobanking remain the lack of human resources, training and a stable budget. It is expected that this WG will be able to provide training, knowledge transfer and networking activities.

Discussion

Based on a table of the main topics touched by the country reports, A. Bilavcik identified three main common requirements that this Working Group (WG) could address:

1) Provide training in cryopreservation
2) Developing or improving protocols for important species/genotypes
3) Setting up collaborative projects.

Another very general issue – the lack of specific funding at the national programme level – was considered an internal problem beyond the possibility of this WG to influence.

Networking

It was pointed out that not all institutes dealing with plant cryopreservation are already involved in this group. Hence it would be ideal to expand the networking activity, for example, to the academic area doing relevant research. The ECPGR Secretary clarified that membership in the WG depends on nominations made by the respective National Coordinators. Current WG members can, in any case, promote and extend collaboration with other colleagues and build national 'cryopreservation communities'. Examples of coordination within national programmes are already existing in the Czech Republic, France, Italy and other countries. M. Faltus expressed the need for this WG to reach out to other ECPGR crop WGs and inform them about the opportunities to promote cryopreservation activities. It was pointed out that a dialogue between WG Chairs is open and recommended (through the dedicated Chairs listserver and/or individual interactions).

It was concluded that the best starting point would be to organize introductory webinars on plant cryopreservation, open to the ECPGR community and beyond. These webinars can be the starting point of a necessary dissemination and communication effort. It would also be useful to understand the needs of the various WGs and verify how this WG could help them.

An important development within ECPGR is the imminent establishment of a new Network of Genebank Managers, which is expected to be a relevant forum for interactions.

Training

Training on garlic is already foreseen as part of the recently approved Garlic-CCS project, within the ECPGR Grant Scheme. This opportunity will however be limited to a small group of selected trainees.

Other training opportunities could be possible outside the ECPGR scheme. This depends on available funding and the availability of experienced labs that are willing to host trainees. Various bilateral programmes are ongoing, offering for example the possibility to validate protocols in different labs. Mobility funds should also be monitored. Group members expressed the need to organize training, for example, on dormant buds' cryopreservation (Portugal, Serbia, Spain) and cryobank standards (Poland). M. Lambardi (Italy) confirmed that CNR could host a training workshop on dormant buds in Italy, which should be organized at the right time, i.e. grafting time at the end of May. He advised that the ongoing COST Action COPYTREE could support training. It is necessary to register in the COST Action and a

proposal could be made to organize the next training in May 2024 on dormant buds[2]. M. Faltus volunteered to contact all the WG members in order to create a list of the existing expertise, interests and needs.

Collaborative project preparation

After informal consultation about prevalent interests within the group, B. Panis proposed to prepare a project proposal to optimize the dormant bud methodology, which is not standardized and often needs to be adapted to local conditions. The project should envisage training, have the purpose of developing a standard protocol, and use accessions representing apple diversity, from which to obtain dormant buds. Volunteers were selected to take the lead in project preparation: M. Höfer, M. Lambardi and A. Bilavcik will start preparing a project and they were encouraged to start with a short concept note. The project can then be further developed for submission to ECPGR, providing the new ECPGR Phase XI will maintain the opportunity to request project funds. Alternatively, it can be submitted to other donors. L. Maggioni encouraged the group to develop a concept for the most rational and efficient way to collaboratively conserve under cryopreservation the relevant European material. A discussion followed with many questions regarding the meaning and scope of 'collaborative action'. M. Faltus confirmed that a concept will start to be developed as part of the Garlic-CCS project, anticipating that a decentralized system will need to be developed and a key factor will be the choice of the priority species/accessions and the guidelines to define them (i.e. national origin, unique diversity testified through molecular analysis, inclusion into the AEGIS European Collection, etc.).

Conclusion

M. Faltus concluded the meeting by thanking all the participants who came to Prague and also the online participants. He was happy to be starting a new era of collaboration in cryopreservation, with the possibility to spread knowledge and improve the safety of the conserved material. B. Panis was also delighted about the collaboration enabled by the WG and the joint planning made, while acknowledging that a lot more needs to be done, since cryopreservation still remains an activity that is not very well known and not widely applied. All the local organizers were warmly thanked for their commitment and excellent organization.

Working Group's Workplan

- *M. Faltus to provide and circulate a frame for the preparation of a 1-page country report to be published in the report of this meeting.*
- *M. Faltus to contact WG members and compile a table of existing expertise, needs and offers to provide training.*
- *M. Faltus and B. Panis to organize introductory webinars on cryopreservation to be offered to the ECPGR Community, with the help of the ECPGR Secretariat.*
- *L. Maggioni to finalize the WG meeting's report on the basis of the offered presentations and country reports to be provided by the WG members.*
- *M. Lambardi to explore the opportunity to organize a COST Action training at CNR, Firenze on*

[2] A Training School on Dormant Bud Cryopreservation was organized in Faenza, Italy, 21-23 May 2024. Details here.

dormant bud cryopreservation in May 2024 and keep the WG informed.

- *M. Höfer, M. Lambardi and A. Bilavcik to prepare a 2-page concept note of a project proposal on droplet vitrification and dormant buds cryopreservation.*

Excursion to the Karlstejn Research Station

After the meeting, the group visited the Viticulture research station of CRI in Karlstejn, where presentations were given of the station operation and grapevine PGR conservation, followed by product testing and dinner.

APPENDICES

Appendix I. Proposal to the ECPGR Executive/Steering Committee for a Cryopreservation WG

The following document, 'Proposal to the ECPGR Executive/Steering Committee for the establishment of a Cryopreservation Working Group', was submitted in October 2021. The proposal was approved by the Steering Committee in February 2022.

Proposal to the ECPGR Executive/Steering Committee for the establishment of a Cryopreservation Working Group

October 29, 2021

Background

Access to a wide range of crop varieties and plant species is essential for maintaining sustainable agriculture. Cross breeding as well as modern plant improvement techniques rely on biodiversity. It is estimated that up to 100,000 plants, representing more than one-third of all the world's plant species, are currently threatened or face extinction in the wild. Their preservation is essential for classical and modern (genetic engineering) plant breeding programmes. Moreover, biodiversity provides a source of compounds to the pharmaceutical, food and crop protection industries.

Storage of dry seeds at low temperature, the most convenient method to preserve plant germplasm, is not applicable to desiccation sensitive seed and vegetatively-propagated plant species. Field collections are not always suitable because germplasm can be easily lost (genetic erosion) due to pests, diseases and adverse weather conditions and maintenance is labour-intensive and expensive. Alternatively, the maintenance of *in vitro* collections is, even under reduced-growth conditions (low-light intensity and low temperature), still labour intensive and there is always the risk of losing accessions due to somaclonal variation, contamination or human error. Cryopreservation or freeze-preservation at ultra-low temperature (-196°C) is the method of choice for long-term conservation, as biochemical and most physical processes are arrested under these conditions.

Advantages of cryopreservation compared to other plant conservation methods are that plant materials are stored (i) genetically stable with no or limited risks for mutations (ii) for a longer term at very low cost and (iii) in disease- and pest-free conditions. Besides its traditional use for the conservation of genetic resources, cryopreservation is now more and more applied for the safe long-term storage of plant tissues with specific characteristics like medicinal and alkaloid-producing cell lines and hairy root cultures, genetically transformed tissues, and transformation-competent tissues. Recently, it was also proven that cryotherapy can be successfully applied to eradicate viruses from a wide range of crops like plum, potato, garlic, apple and grape. However, despite the fact that cryogenic procedures are being developed for an increasing number of recalcitrant seeds and *in vitro* tissues / organs, the routine utilization of cryopreservation for the preservation of plant biodiversity is still limited. This mainly because of the lack of efficient cryopreservation protocols for some important crops and the lack of funding to cryopreserve large collections.

Recently, there is also increasing interest in applying cryopreservation for the long-term (10-100s years) storage of orthodox seeds, especially those that are otherwise inherently short-

lived.

Global Plant Cryopreservation

The importance of cryopreservation for conserving crop genetic resources worldwide can be illustrated by the fact that in June, 2021, a meeting was organised, entitled "Cryopreservation: A long-term strategy for hard-to-conserve PGRFA collections in a post-COVID world", co-organized by the Secretariat of the International Treaty on Plant Genetic Resources for Food and Agriculture and the Global Crop Diversity Trust (http://www.fao.org/plant-treaty/overview/partnerships/international-expert-panel/en/). Moreover, a recent study, "the Feasibility Study for a Safety Back-Up Cryopreservation Facility" (https://www.bioversityinternational.org/fileadmin/user_upload/Feasibility_Acker_2017pdf.pdf), commissioned to investigate and provide recommendations for the long-term secure conservation of recalcitrant seed and clonal crops, stated that "Many of the world's most important crops for food, nutrition and livelihoods, particularly for the poorest people, are vegetatively-propagated or have recalcitrant seeds." The study recognized that a global effort is needed to provide a long-term solution for the conservation of these vital crops so that their genetic diversity can be secured for use by future generations. Following this study, an action plan was developed that includes the establishment of regionally-based specialized Hubs (initially in Europe, Africa, and South America) to act as centres of excellence, with expertise in the long-term conservation of clonal and recalcitrant seed crop genetic resource collections. These Hubs could also serve as important back-up cryopreservation facilities as indicated in the feasibility study. Important European grown crops that would benefit from a facilitated cryopreservation approach are among others potato, pear, apple, garlic, artichoke, berries, grape and hops.

Plant Cryopreservation in Europe

Europe has historically played an important role in developing and promoting cryopreservation for plants. A first initiative was launched within the EU FP5 project "CRYMCEPT": "Establishing Cryopreservation Methods For Conserving European Plant Germplasm Collections" (https://cordis.europa.eu/project/id/QLK5-CT-2002-01279), which ended in October 2005. This project, coordinated by KU Leuven (Belgium) was carried out in collaboration with universities and research institutes from the UK, France, Italy and Germany. The aim was to develop more efficient and generally applicable plant cryopreservation protocols based on fundamental research. In this project, biochemical and physiological aspects of cryopreservation/cryoprotection in a wide variety of plant species was studied in order to unravel cryoprotection. Moreover, at the end of this project two workshops were held with participants from 20 different nationalities who were trained and informed about prospects / applications of plant cryopreservation. A second important European initiative was the EURALLIVEG project (EC 870/2004) conducted between 2007 and 2011. The main focus was the development of a European integrated Allium Core collection provided by national collections of Germany, Czech Republic, Poland, Italy, France, and Nordic countries. This collection was designed as a part of the European Genebank Integrated System (AEGIS) of the European Cooperative Program for Plant Genetic Resources (ECPGR). One of the main aims was the cryopreservation of 200 most important garlic accessions which were finally stored within the Cryobank network of the three partners, i.e. IPK in Germany, CRI in the Czech Republic and RIH in Poland. A third initiative was the EU COST Action CRYOPLANET (see https://www.cost.eu/actions/871/), where cryopreservation techniques for a

wide variety of European plants were developed. This ran from 2006-2011 and involved 21 EU countries. This action resulted in a final publication: "Cryopreservation of Crop Species in Europe" , (https://www.cost.eu/publication/cryopreservation-of-crop-species-in-europe/).

Rationale

According to the feasibility study executed in 2017, eight research institutes (see Table 1) in seven European countries apply cryopreservation as a routine method to conserve their crop genetic resources. These include crops grown in Europe such as apple, citrus, garlic, grape, mint, hops, potato and strawberry, as well as tropical crops important for the European markets such as coffee and banana. Currently about 4,500 accessions are cryopreserved in Europe, almost half of the world's cryopreserved accessions. Nevertheless, many thousands of accessions, including those of apple, citrus, potato, strawberry and other berries, still remain at risk.

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

Proposal

With this, we submit for consideration and approval by the Executive/ Steering Committee of ECP/GR a request for the formal establishment of a Cryopreservation Working Group within the ECPGR. We believe that the formalization of this ECPGR Working Group would be a major step to establish and strengthen collaboration within the entire European region.

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Table 1. European institutes holding (according the feasibility study of 2017) cryopreserved collections

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

Appendix II. Agenda

1st Meeting of the ECPGR Cryopreservation Working Group

Concept for development of a rational and efficient way to collaboratively conserve under cryopreservation the relevant European plant genetic resources

3-4 May 2023

Crop Research Institute, Prague, Czech Republic

Goals:

- Bringing together experts for cryoconservation of plant genetic resources around Europe
- Sharing information about cryopreservation in European countries
- Discussion on strategy for cryoconservation of PGR in Europe
- Discussion on strategy to broaden the routine use of cryopreservation methods

Preliminary Agenda

Tuesday 2 May

Participants' arrival in Prague

Wednesday 3 March

Presentations of participants (*Chairs: B. Panis and M. Faltus*)

09:00 – 09:15	Opening (*CRI Director*)
09:15 – 10:00	Introduction by the co-chairs Milos Faltus and Bart Panis on the working group and cryopreservation in general
10:00 – 10:20	Update on ECPGR (*L. Maggioni*)
10:20 – 10:40	Presentation by *D. Ellis*, topic TBD
10:40 – 11:00	Participants presentations (1)
11:00 – 11:30	*Coffee break*
11:30 – 13:00	Participants presentations (6)
13:00 – 14:00	*Lunch*
14:00 – 16:00	Participants presentations (8)
16:00 – 16:30	*Coffee break*
16:30	Departure to the Viticulture research station of CRI in Karlstejn
17:00 – 21:00	Presentation of the station operation, grapevine PGR conservation, product testing and dinner

Thursday 4 May

Discussion about the concept for a rational and efficient way to collaboratively conserve the relevant European plant genetic resources through cryopreservation
(*Chairs: M. Faltus and B. Panis*)

9:00 – 11:00	General discussion on country reports - Strengths - Weaknesses - Gaps
11:00 – 11:30	*Coffee break*
11:30 – 13:00	Discussion*
13:00 – 14:00	*Lunch*
14:00 – 15:00	Group photo and visit to the genebank and cryobank
15:00 – 15:30	Discussion* (continued)
15:30 – 16:00	*Coffee break*
16:00 – 16:30	Steps needed for the elaboration of the concept, reporting to the ECPGR Steering Committee and future steps
18:00	*Social dinner*

***Discussion topics**

- Other European institutes/countries to be involved
- Need for training? If so, on what topics?
- 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)
- Funding possibilities for collaboration?

Friday 5 May

Departure from Prague

Appendix III. List of participants

Working group members

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Appendix IV. Country reports

BELGIUM

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Bart PANIS	The Alliance of Bioversity International and CIAT, c/o KU Leuven	Tissue culture, Cryopreservation, Seed conservation, gene editing	Developed Cryopreservation protocols for 40 plant species and cryobanking of banana, stevia and sweet potato. Fundamental aspects of cryopreservation
Yordan MUHOVSK	Department Life Sciences Walloon Agricultural Research Centre	in vitro culture, genomics, transcriptomics, proteomics, effectoromics and phytopathology	Agronomically important arable crops and fruit tree species and their relationships with various abiotic and biotic factors, and the molecular mechanisms and functions underlying.

2. List of collections of cryopreserved plant species:

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
The Alliance of Bioversity International and CIAT, c/o KU Leuven	*Musa species*	1200	Shoot tips	Droplet-Vitrification	LN	safety duplication
	Stevia	10	Shoot tips	Droplet-Vitrification	LN	
	Sweet potato	10	Shoot tips	Droplet-Vitrification	LN	
	Edible aroids	6	Shoot tips	Droplet-Vitrification	LN	
	Old chicory land races	422	seeds	Drying+freezing	Vapour phase of LN	

3. Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others

CGIAR, DGD (Belgian Development Cooperation),

4. Is there the national program for the conservation of genetic resources through cryopreservation?

No.

5. Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?

No

6. Is cryopreserved material backed-up in another facility, if so, where?

Yes, the banana collection is backed up as a black box in Montpellier, France.

7. Other putative members for the ECPGR CRYO-WG in your county

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Hannes Wilms	INBO (Research Institute for Nature and Forest - Instituut voor Natuur- en Bosonderzoek)	Tissue culture, Cryopreservation	Coconut, trees droplet-vitrification Research + Cryobanking Forest research Tissue culture
Natalia Sleziak	The Alliance of Bioversity International and CIAT, c/o KU Leuven	Tissue culture, Cryopreservation	Tissue culture Coconut, taro, cassava, sweet potato droplet-vitrification Research + Cryobanking

8. Expectation from CRYO-WG:
Joint projects, technology transfer, establishment of more cryo facilities, fundamental research

9. Training/expertise that can be offered to the WG participants:
Practical cryobanking, droplet vitrification of many plant species, slow freezing of embryogenic cultures, tissue culture

10. Constraints:
Limited funding for cryopreservation

11. Proposals/ideas for a European strategy on plant cryopreservation:
During the last few decades, Europe has been on the forefront of cryopreservation research (FP5 project "CRYMCEPT, Cost action CRYOPLANET, large cryopreserved collections of crop species (potato, garlic, banana, strawberry, …), ….). A new project should bring together those experienced and new researchers to develop new cryopreservation protocols for vegetatively as well as seed propagated species. Maybe a European centre of excellence could be established that provides training and develops cryopreservation protocols.

CZECH REPUBLIC

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Alois BILAVČÍK	Crop Research Institute	Tissue culture, cryopreservation, freezing tolerance, thermal analysis.	fruit trees two-step freezing, ED, vitrification research + cryobanking
Miloš FALTUS	Crop Research Institute	Thermal analysis, tissue culture, cryopreservation.	hop, potato, grapevine droplet-vitrification, simple dehydration research + cryobanking
Jiří ZÁMEČNÍK	Crop Research Institute	Tissue culture, cryopreservation, drought tolerance, thermal analysis.	*Allium* droplet-vitrification research + cryobanking

2. List of collections of cryopreserved plant species:

Institution	Plant Species	No. of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
Crop Research Institute	*Malus domestica* BORK	17	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Pyrus communis* L.	24	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Prunus armeniaca* L.	12	Shot tips	Droplet-Vitrification	LN	safety duplication
Crop Research Institute	*Persica vulgaris* P.MILLER	5	Shot tips	Droplet-Vitrification	LN	safety duplication
Crop Research Institute	*Cerasus avium* L.	3	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Cerasus vulgaris* P.MILLER	10	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Cerasus* P.MILLER	3	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Fragaria* x *ananassa*	34	Shot tips	Encapsulation- Dehydration	LN	safety duplication
Crop Research Institute	*Lonicera* L. (edible)	24	Shot tips	Droplet-Vitrification	LN	safety duplication
Crop Research Institute	*Allium sativum* L.	187	Shot tips	Droplet-Vitrification	LN	safety duplication
Crop Research Institute	*Solanum tuberosum* L.	104	Shot tips	Simple-dehydration	LN	safety duplication
Crop Research Institute	*Vitis vinifera* L.	3	Shot tips	Droplet-Vitrification	LN	safety duplication
Crop Research Institute	*Malus* MILL. <hort. c	6	Dormant buds	Two-step freezing	LN	safety duplication
Crop Research Institute	*Humulus lupulus* L.	68	Shot tips	Simple-dehydration	LN	safety duplication

3. Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others

30% government (institutional), 43% national grant agency, 25% International collaboration, 8% National program of PGR conservation, 0% private, 2% others.

4. Is there a national program for the conservation of genetic resources through cryopreservation?

Crop Research Institute participates in the National program for the conservation of plant genetic resources in the Czech Republic. Our lab is the only responsible participant responsible for cryopreservation activities in the National program.

5. Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?

In addition to vegetatively propagated crops grown in the Czech Republic (especially other fruit trees), collections of recalcitrant species and vegetatively propagated grass can also be safely stored by cryopreservation.

6. Is cryopreserved material backed-up in another facility, if so, where?
A part of the *Allium* collection originated form the EURALLIVEG project has been backed up in the IPK Gaterleben and RIH Skierniewice.

7. Other putative members for the ECPGR CRYO-WG in your county

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Stacy Denise Hammond Hammond	Crop Research Institute	Tissue culture, vitrification method	*Allium*, fruit trees, potatoes, Andean tuber crops

8. Expectation from CRYO-WG:
Experience sharing and transfer, implementation of collaborative projects.

9. Training/expertise that can be offered to the WG participants:
Training in cryopreservation and/or thermal analysis utilization.

10. Constraints:
Unstable funding resulting in unstable experienced staff involving.

11. Proposals/ideas for a European strategy on plant cryopreservation:
Development of small working groups focused on specific problems to be solved (garlic, dormant buds, recalcitrant seeds...)

FINLAND

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Anna Nukari	Natural Resources Institute Finland (Luke)	cryopreservation, tissue culture	fruit trees and berries, hop, *Allium*, modified droplet vitrification research + cryobanking +certified plant production pre-basic mother material preservation
Saija Rantala	Natural Resources Institute Finland (Luke)	cryopreservation	Fruit trees and berries (Ribes), cryo-co-ordination, two-step freezing, database data management, modified droplet vitrification research + cryobanking

2. List of collections of cryopreserved plant species:

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
			(dormant buds/ Shoot tips/seeds/pollen/ ….)	(Two step-freezing, Encapsulation-Dehydration, Droplet-freezing, Vitrification, Droplet-Vitrification, Encapsulation-Vitrification, V cryo-plate, D cryo-plate, another)	(LN, N vapor, refrigerator)	(education, research, breeding, safety duplication, another)
Natural Resources Institute Finland (Luke)	*Fragaria x ananassa*	5	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Hippophaë rhamnoides*	1				
Natural Resources Institute Finland (Luke)	*Malus domestica*	1	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Ribes nigrum* L.	26	dormant buds /Shoot tips	Two step-freezing/ Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Ribes rubrum* group	4	dormant buds	Two step-freezing	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Rubus allegheniensis* Porter	1	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Rubus arcticus*	2	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Rubus idaeus*	9	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Rubus idaeus* x *R. allegheniensis*	2	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Rubus x binatus*	4	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication

VIHRA:						
Natural Resources Institute Finland (Luke)	*Actinidia kolomikta*	1	Dormant buds	Two step-freezing	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Dasiphora fruticosa*	4	Shoot tips	Modified Droplet-Vitrification	LN vapor	safety duplication
Natural Resources Institute Finland (Luke)	*Diervilla x splendens*	1	Dormant buds			
Natural Resources Institute Finland (Luke)	*Diervilla lonicera*	1	Dormant buds			

3. **Sources of financial support for cryopreservation activities:**
   55% government, 33% National program of PGR conservation, 11 % government institutional budget, 0% national grant agency, 0 % private, 0 % EU, others

4. **Is there the national program for the conservation of genetic resources through cryopreservation?**
   Yes, Natural Resources Institute Finland (Luke) is the responsible institute for the national PGR programme and Luke labs are responsible for the cryopreservation research and cryobanking of the national PGR.

5. **Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?**
   Vegetatively propagated crops selected as national mandates, especially fruits and berries are being prioritized

6. **Is cryopreserved material backed-up in another facility, if so, where?**
   Back-up tanks at two separate locations inside Luke, some parts of the collections divided between the locations, transfer between tanks on-going

7. **Other putative members for the ECPGR CRYO-WG in your county**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking

8. **Expectation from CRYO-WG:**
   Expertise & cryobanking best practices sharing: Discussion on strategy for cryoconservation of PGR at European and species-specific levels, AEGIS, standards, back-ups, databases, prioritization, protocols, health status & cryotherapy

9. **Training/expertise that can be offered to the WG participants:**
   Visits to Luke cryopreservation labs

10. **Constraints:**
    Qualified experienced staff available in varying degrees

11. **Proposals/ideas for a European strategy on plant cryopreservation:**
    Discussion on strategy for cryobanking of PGR at European and species-specific levels

ITALY

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Maurizio Lambardi	National Research Council (CNR), IBE-Institute of BioEconomy, Sesto Fiorentino (Firenze), Italy	Tissue culture, micropropagation, cryopreservation	Woody plants Dormant bud technique Research + cryobanking
Emilia Caboni	Council for Research in Agriculture and the analysis of the agricultural economy (CREA) - Olive, Fruit and Citrus Crops Research Center (OFA), Rome, Italy	Tissue culture, micropropagation, cryopreservation	Fruit species. Development of encapsulation-dehydration and droplet vitrification axillary bud techniques and related research
Claudia Ruta	Department of Soil, Plant and Food Sciences, University of Bari Aldo Moro, Bari, Italy	Tissue culture, micropropagation, cryopreservation	Vegetable and Pulses species. Development of cryopreservation techniques and related research Cryobanking

2. List of collections of cryopreserved plant species

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
CNR-IBE	*Citrus* spp.	24	Polyembrionic seeds	Seed dehydration	LN	Safety duplication
CNR-IBE	*Malus domestica*	19	Uni-nodal scions	Dormant bud technique	LN	Safety duplication
CNR-IBE	*Prunus domestica*	8	Uni-nodal scions	Dormant bud technique	LN	Safety duplication
UNIBA-DiSSPA	*Pulses*	20	Seeds	Seed dehydration	LN	Safety duplication
UNIBA-DiSSPA	*Cicer arietinum*	2	Shoot tips	PVS2-Vitrification	LN	Safety duplication

3. Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others

CNR-IBE

20% government (institutional), 40% National program of PGR conservation, 35% EU project, 5% collaboration with private organizations.

CREA

80% National program of PGR conservation, 20% EU project.

UNIBA

100% Regione Puglia Administration under Rural Development Measure 10.2.1 PSR Puglia Programming cycles 2007-2013, 2014-2022 "Program for the conservation and the valorization of the genetic resources in agriculture". Project "Biodiversity of vegetable crops in Puglia (BiodiverSO)", Project "SaveGrainPuglia".

4. Is there the national program for the conservation of genetic resources through cryopreservation?

CNR-IBE and CREA-OFA participate in the ''International Treaty on Plant Genetic Resources for Food and Agriculture - RGV-FAO, 2023-2025'' from the Italian Ministery of Agriculture, Food and Forests.

5. Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?

The "BioMemory Project": BioMemory is the network of biological collections of the

Department of Biology, Agriculture and Food Sciences (DiSBA) of the National Research Council of Italy (CNR) for bio-monitoring, biodiversity conservation, agri-food and environmental sustainability, and human well-being (https://biomemory.cnr.it/).
The "PLANTA*res*" network: National Network on Plant Genetic Resources for Food and Agriculture realized within the project "Plant Genetic Resources/FAO" (RGV/FAO), financed by the Ministry of Agriculture, Food and Forests. Project partners are the Research Centres and Units of the CREA, the "Istituto di Bioscienze e Biorisorse" (IBBR - Mediterranean Germplasm Database) of the CNR in Bari, and the NGO "Rete Semi Rurali".

6. Is cryopreserved material backed-up in another facility, if so, where?
No.

7. Other putative members for the ECPGR CRYO-WG in your country

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Doaa Elazab Elkassas	National Research Council (CNR), IBE-Institute of BioEconomy, Sesto Fiorentino (Firenze), Italy	Tissue culture, micropropagation, cryopreservation	Hazelnut, banana Droplet vitrification Research

8. Expectation from CRYO-WG:
Experience sharing and transfer, implementation of collaborative projects.

9. Training/expertise that can be offered to the WG participants:
Training Course on the Dormant Bud Technique

10. Constraints:
Lack of generational turnover in the cryopreservation laboratory and, therefore, the risk of losing the skills acquired over time

11.Proposals/ideas for a European strategy on plant cryopreservation:
To create an increasingly cohesive European group of cryopreservation, in order to be able to exchange and compare the skills acquired by the different teams, as well as to have more planning strength in proposing projects and achieving European funds.

NORWAY

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Zhibo Hamborg	Norwegian Institute of Bioeconomy Research (NIBIO)	Cryopreservation Plant virus	Research activities on cryopreservation and healthy plant materials

2. List of collections of cryopreserved plant species:

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
			(dormant buds/ Shoot tips/seeds/pollen/….)	(Two step-freezing, Encapsulation-Dehydration, Droplet-freezing, Vitrification, Droplet-Vitrification, Encapsulation-Vitrification, V cryo-plate, D cryo-plate, another)	(LN, N vapor, refrigerator)	(education, research, breeding, safety duplication, another)
Sagaplant AS	*Fragaria x ananassa*	7	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Fragaria ssp.*	31	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Malus domestica*	28	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Prunus domestica*	1	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Ribes nigrum*	7	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Rubus fruticosus*	3	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Rubus idaeus*	19	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Rubus occidentalis*	1	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication
Sagaplant AS	*Solanum tuberosum*	47	Shot tips	Droplet-Vitrification	LN	Conservation/safety duplication

3. Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others

- The Norwegian Agriculture Agency is administrating a financial instrument to support stakeholders in the field of PGRFA conservation and use. Sagaplant has applied and received funding to partly cover the cost of the conservation activities.
- Funding through research grants (national and European) has been important to further develop the cryopreservation activities in Norway, including development of new protocols.

4. Is there the national program for the conservation of genetic resources through cryopreservation?

There is a national program for the conservation of PGRFA, including one facility (Sagaplant

AS) were approximately 144 accessions are conserved through cryoconservation. Sagaplant AS is a national plant health center and is producing elite plants and planting material of strawberries, raspberries and fruit trees to Norwegian producers. Sagaplant is also specialized in cryopreservation of plant genetic material. The company started experimenting with cryotechnology in 2007 and has in collaboration with the Norwegian Institute of Bioeconomy Research (NIBIO) received funding to develop the cryo-storage methods further. In 2014 systematic conservation of berries and potato varieties started and in 2023 there were 144 accessions of 9 different species in cryo-storage.

5. **Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?**

Yes, the Norwegian Genetic Resources Centre is responsible for the national inventory. It is updated every year and is available online: https://www.nibio.no/tema/mat/plantegenetiske-ressurser/bevaring-av-plantemateriale/bevaring-i-klonarkiv-feltgenbank/planteregister?locationfilter=true

6. **Is cryopreserved material backed-up in another facility, if so, where?**

Yes, the cryopreserved material of fruits and berries are conserved as living plants in various clonal collections in Norway. The main collection of potato accessions is conserved *in vitro* at NIBIO's Division of Biotechnology and Plant Health at Ås.

7. **Other putative members for the ECPGR CRYO-WG in your county**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Tor Skilbred	Sagaplant AS	Manager	Conservation of genetic resources, cryoconservation

8. **Expectation from CRYO-WG:**

- Knowledge and personal exchange
- Collaboration

9. **Training/expertise that can be offered to the WG participants:**

10. **Constraints:**

- Capacity and funding for developing conservation techniques.
- Efforts to identify and document all the accessions in the ex situ collections and prioritize accession to be conserved through cryopreservation.

11. **Proposals/ideas for a European strategy on plant cryopreservation:**

POLAND

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Karolina TOMICZAK	Plant Breeding and Acclimatization Institute - National Research Institute in Radzików (PBAI-NRI in Radzików)	Tissue culture, cryopreservation, analysis of genetic stability using molecular markers and flow cytometry	• *Gentiana*, fern gametophytes and somatic embryos, potato • Encapsulation-dehydration, encapsulation-vitrification, vitrification, droplet-vitrification • Research + initiation of cryobanking

2. List of collections of cryopreserved plant species:

Institution	Plant Species / Genus	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
The Kostrzyca Forest Gene Bank (Kostrzyca FGB)	*Abies* alba Mill.	307	seeds	direct freezing	LN	safety duplication
	Alnus alnobetula (Ehrh.) K.Koch	1	seeds	direct freezing	LN	safety duplication
	Alnus glutinosa (L.) Gaertn.	264	seeds	direct freezing	LN	safety duplication
	Betula humilis Schrank	3	seeds	direct freezing	LN	safety duplication
	Betula nana L.	8	seeds	direct freezing	LN	safety duplication
	Betula pendula var. *oycowiensis* Roth	164	seeds	direct freezing	LN	safety duplication
	Betula pendula var. *oycowiensis* (Besser) Dippel	1	seeds	direct freezing	LN	safety duplication
	Carpinus betulus L.	1	seeds	direct freezing	LN	safety duplication
	Daphne mezereum L.	1	seeds	direct freezing	LN	safety duplication
	Dictamnus albus L.	2	seeds	direct freezing	LN	safety duplication
	Frangula Mill.	1	seeds	direct freezing	LN	safety duplication
	Frangula alnus Mill.	1	seeds	direct freezing	LN	safety duplication
	Fraxinus excelsior L.	98	seeds	direct freezing	LN	safety duplication
	Fraxinus excelsior L.	47	dormant buds	programmed freezing	LN	safety duplication
	*Hippophae rhamnoides*L.	1	seeds	direct freezing	LN	safety duplication
	Malus sylvestris (L.) Mill.	1	seeds	direct freezing	LN	safety duplication
	Pinus × *rhaetica* Brügger	2	seeds	direct freezing	LN	safety duplication
	Prunus avium (L.) L.	28	seeds	direct freezing	LN	safety duplication
	Prunus fruticosa Miyoshi	1	seeds	direct freezing	LN	safety duplication
	Prunus spinosa L.	1	seeds	direct freezing	LN	safety duplication
	Quercus petraea (Matt.) Liebl.	34	plumules	vitrification	LN	safety duplication, research
	Quercus robur L.	81	plumules	vitrification	LN	safety duplication, research
	Rhododendron ferrugineum L.	1	seeds	direct freezing	LN	safety duplication
	Rhododendron luteum Sweet	2	seeds	direct freezing	LN	safety duplication
	Salix lapponum L.	3	seeds	direct freezing	LN	safety duplication
	Sambucus nigra L.	1	seeds	direct freezing	LN	safety duplication
	Sorbus aria (L.) Crantz	1	seeds	direct freezing	LN	safety duplication
	Sorbus aucuparia L.	1	seeds	direct freezing	LN	safety duplication
	Sorbus sudetica (Tausch) Bluff, Nees & Schauer	2	seeds	direct freezing	LN	safety duplication
	Sorbus torminalis (L.) Crantz	4	seeds	direct freezing	LN	safety duplication
	Taxus baccata L.	2	seeds	direct freezing	LN	safety duplication
	Tilia cordata Mill.	34	seeds	direct freezing	LN	safety duplication
	Ulmus glabra Huds.	94	seeds	direct freezing	LN	safety duplication
	Ulmus laevis Pall.	66	seeds	direct freezing	LN	safety duplication
	Ulmus minor Mill.	2	seeds	direct freezing	LN	safety duplication

	237 rare and endangered herbaceous species (from 41 families), growing in Polish forests and meadows https://www.lbg.lasy.gov.pl/zachowanie-lesnych-zasobow-genowych-gatunki	451	seeds	direct freezing	LN	safety duplication
The Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin (PAS BG-CBDC in Powsin)	*Gentiana capitata* Buch.-Ham. ex D.Don	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Gentiana cruciata L.	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Gentiana decumbens L.f.	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Gentiana kurroo Royle	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Gentiana pannonica Scop.	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Gentiana tibetica King ex Hook.f.	1	proembryogenic mass	encapsulation-dehydration	LN	research
	Malus domestica Borkh.	289	dormant buds	programmed freezing	LN	safety duplication
	Asplenium adulterinum Milde	1	gametophytes	encapsulation-dehydration	LN	research
	Asplenium adiantum-nigrum L.	1	gametophytes	encapsulation-dehydration	LN	research
	Asplenium cuneifolium Viv.	1	gametophytes	encapsulation-dehydration	LN	research
	Asplenium scolopendrium L.	1	gametophytes	encapsulation-dehydration	LN	research
	Ceratopteris cornuta (P. Beauv.) Lepr.	1	gametophytes	encapsulation-dehydration	LN	research
	Ceratopteris thalictroides (P. Beauv.) Lepr.	1	gametophytes	encapsulation-dehydration	LN	research
	Cibotium glaucum (Sm.) Hook. & Arn.	1	gametophytes	encapsulation-dehydration	LN	research
	Cibotium schiedei Schltdl. & Cham.	1	gametophytes	encapsulation-dehydration	LN	research
	Cyathea australis Domin.	1	gametophytes	encapsulation-dehydration	LN	research
	Cyathea dealbata (G. Forst.) Sw.	1	gametophytes	encapsulation-dehydration	LN	research
	Cyathea delgadii Sternb.	1	gametophytes	encapsulation-dehydration	LN	research
	Alsophila smithii (Hook.f.) R.M.Tryon	1	gametophytes	encapsulation-dehydration	LN	research
	Dicksonia fibrosia Colenso	1	gametophytes	encapsulation-dehydration	LN	research
	Dicksonia sellowiana Hook.	1	gametophytes	encapsulation-dehydration	LN	research
	Osmunda regalis L.	1	gametophytes	encapsulation-dehydration	LN	research
	241 rare and endangered herbaceous species of Polish flora	885	seeds	direct freezing	LN	safety duplication
Institute of Horticulture – National Research Institute in Skierniewice (InHort Skierniewice)	*Allium sativum* L.	228	shoot tips isolated from bulbils (bolting forms) or cloves (non-bolting forms)	vitrification	LN	safety duplication
Plant Breeding and Acclimatization	*Solanum tuberosum* L. 4*x*	4	shoot tips	vitrification	LN	safety duplication, research

Institute – National Research Institute, Młochów Division (PBAI-NRI, Młochów Division)	*Solanum tuberosum* L., hybrids 2*x*	56	shoot tips	vitrification	LN	safety duplication
	Solanum tuberosum L., hybrids 2*x*	96	pollen	direct freezing	LN	safety duplication
	Solanum phureja Juz. et Buk.	4	pollen	direct freezing	LN	safety duplication
	Solanum kurtzianum Bitter & Wittm.	2	pollen	direct freezing	LN	safety duplication
	Solanum ruis-ceballosi Card.	3	pollen	direct freezing	LN	safety duplication
	Solanum pinnatisectum Dunal	4	pollen	direct freezing	LN	safety duplication
	Solanum michoacanum (Bitter) Rybd.	7	pollen	direct freezing	LN	safety duplication

3. **Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others**

European Union:

- the establishment of the cryogenic collections of seeds of rare and endangered plant species at the PAS BG-CBDC in Powsin and at the Kostrzyca FGB (via different operational programmes and environmental projects)
- the establishment and the expansion of the cryogenic collection of forest species at the Kostrzyca FGB (via PHARE)
- the establishment and the expansion of the cryogenic collection of *Allium* at InHort Skierniewice (via EURALLIVEG project)

National grant agencies:

- The establishment of the cryogenic collections of *Malus*, *Gentiana* and ferns at the PAS BG-CBDC in Powsin

Government:

- the establishment of the cryogenic collection of *Solanum* at the PBAI-NRI, Młochów Division
- the expansion of the collection of the cryogenic collection of *Allium* at InHort Skierniewice
- the expansion of the collection of *Malus* sp. at the PAS BG-CBDC in Powsin

The State Forests:

- the establishment and the expansion of the cryogenic collections of forest species at the Kostrzyca FGB

Others (World Bank):

- the establishment of the Kostrzyca FGB

4. **Is there the national program for the conservation of genetic resources through cryopreservation?**

There is no separate program for the conservation of plant genetic resources through cryopreservation in Poland. The cryogenic collections of *Malus* at the PAS BG-CBDC in Powsin, *Solanum* at the PBAI-NRI, Młochów Division, and *Allium* at InHort Skierniewice are maintained within the framework of a National Crop Plant Genetic Resources Protection Program, determined by the Minister of Agriculture and Rural Development. The program is implemented and coordinated by the National Centre for Plant Genetic Resources: Polish Genebank (NCPGR), a department of the Plant Breeding and Acclimatization Institute – National Research Institute in

Radzików. The Kostrzyca FGB implements another program, i.e., the Program of Forest Genetic Resources Preservation and Selection Breeding of Forest Trees. The two cryogenic seed collections of rare and endangered plant species at Kostrzyca FGB and at the PAS BG-CBDC in Powsin are a result of environmental projects financed mainly by the European Union, within the framework of the Operational Programme Infrastructure and Environment.

5. **Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?**
   The Potato Gene Bank, located in the Bonin Division of the Plant Breeding and Acclimatization Institute - National Research Institute, maintains an in vitro collection of over 1500 healed potato cultivars and breeding lines from domestic and world potato collections. This collection is planned to be successively introduced into liquid nitrogen.

6. **Is cryopreserved material backed-up in another facility, if so, where?**
   A part of the *Allium* collection originated form the EURALLIVEG project has been backed up in the IPK Gatersleben (Germany) and Crop Research Institute (Prague, Czech Republic).

7. **Other putative members for the ECPGR CRYO-WG in your county**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Maria BURIAN	Institute of Horticulture – National Research Institute in Skierniewice (InHort Skierniewice)	Plant tissue culture, cryopreservation, analysis of metabolic activity under stress conditions using various methods determining content of metabolites related to ROS	• *Allium, Arabidopsis, Fragaria, Rubus, Lactuca, Capsicum, Raphanus, Armoracia, Rheum, Solanum, Beta, Smallanthus, Daucus* • Vitrification, Encapsulation-dehydration, Encapsulation-vitrification, Droplet-vitrification, Anther *in vitro* culture, Isolated microspore culture in *in vitro* conditions, Plasma membrane isolation, Isolation of cell-wall protein fraction, Immunobloting analyses, RT-PCR analyses, The enzyme-linked immunosorbent assay (ELISA), Preparation of plant tissue material for microscopic analyses using microtome, Extraction of apoplastic fluid, Blue Native electrophoresis of plasma membrane proteins, Determination of hydrogen peroxide concentration, Ascorbate and glutathione assay, Histochemical staining of ROS, Lipid peroxidation level estimation, Measurement of various enzymatic activity • Research + initiation of cryobanking

8. **Expectation from CRYO-WG:**
   Sharing the experience (especially in the field of cryobank management and setting standards), joint workshops, the implementation of collaborative projects

9. **Training/expertise that can be offered to the WG participants**
   - only cryopreservation of seeds of forest species at this point

10. Constraints:

- poor and unstable funding, especially poor employee financing, which results in serious problems with the recruitment and retention of experienced and qualified employees
- an inadequate implementation of cryogenic techniques for the storage of crop germplasm
- a lack of a central cryobank in the National Centre for Plant Genetic Resources: Polish Genebank (NCPGR)

11. Proposals/ideas for a European strategy on plant cryopreservation:

The formation of small working groups focused on specific problems, as suggested by colleagues from the Czech Republic, is a good idea.

PORTUGAL

1. **National representatives and their expertise**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Octávio SERRA	National Institute for Agrarian and Veterinarian Research (INIAV) - BPGV	Tissue culture, Cryopreservation, Genomics	*Allium*, Fruit trees Vitrification, droplet-vitrification Research + Cryobanking

2. **List of collections of cryopreserved plant species:**

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
National Institute for Agrarian and Veterinarian Research (INIAV) - BPGV	*Allium sativum L.*	40	Shoot tips and immature inflorescence bases	Vitrification, Droplet-Vitrification	LN	research, safety duplication

3. **Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others**
   100% government (Institutional).

4. **Is there the national program for the conservation of genetic resources through cryopreservation?**
   There is a National program for the conservation of plant genetic resources in Portugal, and the Portuguese genebank (BPGV) has been involved in the National program since its inception. BPGV is responsible for the conservation of PGR in cold storage, field collections, in vitro and also through cryopreservation.

5. **Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?**

   Yes. All national collections of vegetatively propagated species are well identified and inventoried. These species are potentially cryopreservable.

6. **Is cryopreserved material backed-up in another facility, if so, where?**
   No. The material is currently stored at the national genebank facilities (BPGV) only.

7. **Other putative members for the ECPGR CRYO-WG in your county**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Isabel SILVA	National Institute for Agrarian and Veterinarian Research (INIAV)-BPGV	Tissue culture, Cryopreservation	*Allium, Humulus lupulus*, Fruit trees Vitrification, droplet-vitrification Research + Cryobanking

8. **Expectation from CRYO-WG:**
   Knowledge transfer and training from experienced members, identification of synergies and interests for joint projects

9. **Training/expertise that can be offered to the WG participants:**

Not enough expertise in cryopreservation to offer to other WG members

10. **Constraints:**
Limited expertise and lack of dedicated funding for cryopreservation

11. **Proposals/ideas for a European strategy on plant cryopreservation:**
European countries should identify their national priorities regarding plant cryopreservation, mainly the species or groups of species to be cryopreserved and the techniques to be used. In the scope of this working group, those countries with similar priorities should work together and develop new synergies that benefit all. Initially this would translate into joint research projects that should contribute for the increase of plant cryopreservation activities. Later, at the cryobanking level, those countries could exchange cryopreserved PGR in order to increase the safety of duplicates, similar to what is done for PGR conserved as seed in cold storage.

SERBIA

1. National representatives and their expertise

Name	Institution	Expertise	Activities – species, methods, research / cryobanking
Darko Jevremović	Fruit Research Institute, Čačak, Serbia	plant pathology, cryotherapy	Detection of pathogens of continental fruit species, evaluation of efficiency of cryotherapy, thermotherapy and chemotherapy
Tatjana Vujović	Fruit Research Institute, Čačak, Serbia	tissue culture, cryopreservation, evaluation of gross genetic stability of in vitro plants (determination of chromosome number...)	Continental fruit species, Encapsulation dehydration, vitrification, droplet-vitrification, V cryo-plate, D cryo-plate, pollen cryopreservation
Slađana Jevremović	University of Belgrade, Institute for Biological Research "Siniša Stanković" National Institute of the Republic of Serbia	Tissue culture, cryopreservation, oxidative stress, genetic transformations	Ornamental and medicinal plants, Encapsulation dehydration, vitrification, droplet-vitrification, V cryo-plate, D cryo-plate

2. List of collections of cryopreserved plant species:

Institution	Plant Species	No of accessions	Plant Material	Cryopreservation Method	Storage Condition	Status / Purpose
University of Belgrade, Institute for Biological Research "Siniša Stanković" National Institute of the Republic of Serbia	*Viola cornuta* transgenic lines	15	Shoot tips	Vitrification	LN	Unique collection
University of Belgrade, Institute for Biological Research "Siniša Stanković" National Institute of the Republic of Serbia	*Aesculus flava*	12	Cell suspensions	One-step freezing, Encapsulation dehydration	LN	Unique collection

Comment: Due to a lack of financial support, we still do not have a cryopreserved collections of any fruit plant species for which we have developed cryopreservation protocols. We only have short-term *in vitro* collections.

3. Sources of financial support for cryopreservation activities: government, national grant agency, private, EU, others

We currently do not have any specific financial support for cryopreservation activities. Previously, we had a project titled 'CryoPlum,' which financed only research activities devoted to the optimization of protocols for autochthonous plum cryopreservation. However, we are still conducting research funded by the grant from the Ministry of Science, Technological Development, and Innovation of the Republic of Serbia.

4. Is there the national program for the conservation of genetic resources through cryopreservation?

No.

5. Is there a national inventory for vegetatively propagated plant material (potentially cryopreservable)?

Yes, there is. Currently, three research institutions in Serbia (Faculty of Agriculture – University of Belgrade; Faculty of Agriculture – University of Novi Sad; and Fruit Research Institute, Čačak) are involved in conservation efforts, both in situ and ex situ, with the largest collection of autochthonous genotypes located at the Fruit Research Institute in Čačak. The collections are maintained in open fields and screen

houses, serving multiple purposes including conservation, propagation, exchange, morphological characterization, and agronomic evaluation.

6. **Is cryopreserved material backed-up in another facility, if so, where?**
   No**.**

7. **Other putative members for the ECPGR CRYO-WG in your county**

Name	Institution	Expertise	Activities – species, methods, research / cryobanking

8. **Expectation from CRYO-WG:**
   Experience sharing and transfer, implementation of collaborative projects.

9. **Training/expertise that can be offered to the WG participants:**
   Training in cryopreservation and/or pathogen detection methods.

10. **Constraints:**
    Lack of funding.

11. **Proposals/ideas for a European strategy on plant cryopreservation:**
    A large European project on cryopreservation aims to establish a centralized European cryobank for fruit species. Each partner will develop a protocol for their most important autochthonous genotypes and deposit specimens into the bank.

SLOVENIA

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Slovenia has a program for the conservation of gene resources for agronomically important plants, the Slovenian National Plant Genebank Program (SRGB). The SPGB program is responsible for establishing plant genetic bank collections (https://spletni2.furs.gov.si/srgb/srgb.htm) and is coordinated by the Ministry of Agriculture, Forestry and Food and the Agricultural Institute of Slovenia. The SRGB includes accessions from both public and private collections. In 2017, the database included 5440 accessions of 248 plant species (Grižon, 2012, Šuštar Vozlič, Meglič, 2017).
Although Slovenia has a plant genetic resources program that includes permanent plantings and *in vitro* collections of various agronomically important plants, there are currently no activities for long-term cryopreservation of clonally propagated agronomically important plants in particular, although there are some potential future plans.
Current Slovenian cryopreservation activities are related to conservation of wild species, especially endangered, rare, or vulnerable wild species. Cryopreservation should be one of several activities for their conservation. The activities at the Department of Biology, Faculty of Natural Sciences and Mathematics (FNM), University of Maribor started in 2011 with joining the COST Action 871 with the rare endemic wild species *Hladnikia pastinacifolia*. *Hladnikia* is a monotypic endemic genus with an extremely small range of only 4 km2 in the Trnovski gozd plateau (Slovenia). In early 2010, an efficient protocol for rapid propagation of *Hladnikia in vitro* for *ex situ* conservation was developed (Ciringer et. al. 2016, Ambrožič-Dolinšek et al. 2017). The development of these protocols enables research on cryopreservation. Two methods have been tested and optimised: Encapsulation-dehydration and Encapsulation-vitrification for cryopreservation of meristem or small shoots of *Hladnikia* (Ciringer et. al. 2018, AmbrožičDolinšek et al. 2022). Since 2011, we have had several facilities and equipment for developing cryopreservation protocols, but no facilities for long-term storage. We started developing protocols for slow growth.
After the first meeting of the ECPGR cryopreservation working group in Prague, we contacted Andreja Černak (andreja.cerenak@ihps.si , IHPS) and Nataša Štajner (Natasa.Stajner@bf.unilj.si, Chair of Genetics, Biotechnology, Statistics and Plant Breeding, Faculty of Biotechnology, University of Ljubljana, Ljubljana) and informed them about the possibilities of joining this working group. Nataša is interested in cryotherapy and virus elimination in the future.

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