**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