New AEGIS Project – Genebank Peer Review Report

Place: Genebank Department in Czech Agrifood Research Center (CARC), Prague, Czech Republic

Date: May 19, 2025

<u>Participants Genebank Department in CARC</u>: Dagmar Janovská, Ludmila Papoušková, Vojtěch Holubec,

Alois Bilavčík

Reviewers: Tamar Jinjikhadze (LEPL), Levan Ujmajuridze (LEPL), Silvia Străjeru (SVGB)

Background

Within the framework of the New AEGIS project, the methodology initially developed under the AGENT project is being further implemented. This initiative focuses on assessing the operations of European genebanks and supporting their improvement through a structured system of reciprocal visits and collaborative efforts. The approach is designed to serve as a model for broader implementation across the European genebank network. As part of this initiative, experts from nine genebanks will conduct reciprocal visits to evaluate operational efficiency using jointly developed protocols. These evaluations will result in detailed reports containing recommendations for improvement, which will also be used to engage funding agencies to strengthen specific capacities. The third cycle of this peer review process includes the genebanks of the Czech Agrifood Research Center (CARC) in the Czech Republic, the Scientific Research Center of Agriculture (LEPL) in Georgia, and the "Mihai Cristea" Vegetal Genetic Resources Bank (SVGB) in Romania.

Visit and Organization

The third genebank visit in this cycle was conducted at the Genebank of the Czech Agrifood Research Center (CARC) in Prague, the Czech Republic. During the visit, the Genebank's manager, Dagmar Janovská, provided an overview of the National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity (NPPGRFA). Comprehensive information was shared regarding the Genebank's mission, core activities, funding mechanisms, and human resources framework. A guided tour of the Genebank's facilities, including seed storage, *in vitro* preservation, and cryopreservation units, was conducted. Key activities were presented, and all questions raised during the visit were addressed. As an additional resource, the Genebank Operational Manual, updated in March 2025, was utilized to provide further insights into the Genebank's operations.

Overview of the CARC Genebank

The CARC Genebank functions as the central facility for the conservation of generatively propagated crops in the Czech Republic. It operates under NPPGRFA, which was established by the Czech Ministry of Agriculture in 1993. The genebank team at CARC coordinates NPPGRFA, which covers all crops relevant to food and agriculture in the Czech Republic. The NPPGRFA is a national network comprising 12 crop-focused research institutes, one university, and several companies across 15 different locations. CARC hosts the central genebank for all generatively propagated crops, representing 81 % of all species maintained within the national PGRFA collections. In addition, a cryobank in CARC has been established for the safety duplication of vegetatively propagated plant material.

Quality Management and Compliance

To maintain high operational standards, the Genebank has been following the ISO 9001:2015 Quality Management System for its seed genebank activities since 2011. This system is certified by TÜV SÜD Czech Ltd., with the current certification valid until 2025. Additionally, the Genebank adheres to Act No. 148/2003, which governs the conservation and utilization of plant genetic resources. The Standard Material Transfer Agreement (SMTA) has been utilized for seed transfers since 2012, including all, not only those involving Annex I crops, ensuring thorough documentation and compliance with international reporting requirements.

Collaborative Agreements and Data Management

The Genebank has established formal agreements with the Slovak genebank and the Svalbard Global Seed Vault for the safety duplication of germplasm collections, thereby enhancing the security of its resources. However, only a small portion of the genetic fund is duplicated either in Slovak genebank (3,346 accessions) or in Svalbard (1,973 accessions). For data management, the Genebank employs the GRIN Czech system, which is based on GRIN Global, to manage and share passport, characterization, evaluation, and distribution data. This data is publicly accessible online and is shared annually with EURISCO, ensuring adherence to global information exchange standards.

Recommendation 1. - To mitigate the risk of genetic resource loss in the event of unforeseen disasters, the Genebank should aim to duplicate a larger proportion of its accessions. Best practices suggest maintaining at least one duplicate of each accession as a safety backup.

Recommendation 2. - Implement a routine schedule for duplicating accessions

Germplasm management

Seed collections

The CARC Genebank employs comprehensive practices for seed management, ensuring the conservation of plant genetic resources collections under optimal conditions. Seed samples obtained from cooperating institutes are temporarily stored at +5°C until space becomes available in the drying chamber. All crops whose seeds are stored in the central genebank are processed – including drying and other preparatory steps – in accordance with procedures defined in the ISO documentation.

Seeds undergo drying in a chamber maintained at +17 - 20°C and 14% relative humidity to achieve moisture levels between 3 % and 9 %, depending on species requirements. Only high-quality seeds that are fully ripe, pure, healthy, and viable are accepted into genebank storage. Samples that fail to meet these standards are returned to curators along with detailed error reports.

The genebank maintains its active and base collections at -18°C, which ensures optimal conditions for long-term storage. Seeds are stored in glass containers with twist-sealed lids and silica gel packets to control moisture, with moisture levels maintained between 3% and 9%, depending on the species. Seeds for the active collection are stored without creating sub-samples, with glass containers opened as needed for distribution. For AEGIS accessions, sub-samples are prepared in plastic bags and stored alongside silica gel in glass jars. All seed samples are tagged with barcodes for identification.

The genebank currently operates six out of ten storage chambers, accommodating 45,000 accessions in 97,000 containers, representing 95 % of the generatively propagated crop collections within the National Programme. The facility's capacity is 60 % utilized, leaving room for future expansion.

Viability monitoring is conducted systematically, with a recommended frequency of 10 years for most accessions due to the long-term storage conditions at -18°C. This interval is extended to 20-25 years for cereal crops, which account for about 50% of stored accessions. The viability monitoring schedule is influenced by the initial quality and quantity of the seed lot. Samples with low seed quantities or low germination trigger notifications for curators to regenerate and replenish stocks. Viability data, germination levels, and seed quality status are recorded and made accessible to curators via the GRIN Czech database, where flagged samples indicate the need for regeneration. After regeneration, different seed lots of the same accession are not mixed but stored in parallel to maintain traceability.

Recommendation 3. - For base collection and safety-duplication, it is advisable to transition to lighter and less fragile materials than glass, such as aluminium foil bags, which offer excellent moisture and air barrier properties and are more space-efficient when used under vacuum conditions. For active collections (frequently accessed materials), glass jars may remain a suitable option due to their durability and air-tight properties.

Recommendation 4. - To minimize the risk of errors in accession handling and enhance overall efficiency in the germplasm management process, it is essential to prioritize the implementation of handheld barcode readers or mobile scanning devices.

Cryopreserved collections

The Cryobank was established as the safety duplication for vegetatively propagated crops involved in NPPGRFA. The cryobank houses 534 accessions across 14 species and has implemented stringent measures to ensure the high viability of cryopreserved explants. Healthy, contamination-free plant material is used with cultivation media tailored to species-specific requirements based on the Murashige and Skoog (MS) medium. Plant material, such as *Allium* bulbils, must be fully matured, while woody species are processed during the eco-dormant stage. Virus elimination procedures have shown a limited impact on regeneration rates in *Allium* species after cryopreservation.

Viability monitoring involves random selection assessments conducted at three- and ten-year intervals, supported by a specialized Excel-based system for species- and accession-specific decisions. The facility sets a minimum regenerative capacity of 30% for successful cryopreservation and requires at least 120 plant meristems to be stored in liquid nitrogen. Accessions with low regeneration rates undergo repeated cryopreservation to improve recovery chances.

Plant samples are stored in liquid nitrogen using cryovials, cryotubes or plastic bags, with manual monitoring of nitrogen levels in Dewar flasks. Cryoprotocols are species-specific, often requiring genotype-based modifications, and favour vitrification solutions without DMSO to minimize mutagenesis risks. Virus-infected and virus-free materials are not segregated, as cross-contamination is deemed unlikely.

The Cryobank serves primarily as a duplicate collection, with a minimum of 120 shoot tips stored per accession.

Recommendation 5. - Consider raising the minimum regenerative capacity threshold above 30 % for successful cryopreservation. This could help ensure that only the most viable plant materials are stored, thereby improving overall recovery rates.

Funding and Financial Stability:

Increasing Baseline Funding

Recommendation 6. - Advocate for higher government funding to reduce reliance on external projects and ensure the sustainability of core operations.

Conclusion

The Genebank at the Czech Agrifood Research Center demonstrates a solid basis for the conservation of plant genetic resources and offers considerable potential for further development. Opportunities such as expanding the duplication of accessions, strengthening staff capacity and securing additional funding would further enhance Genebank's resilience and operational efficiency. By building on its existing strengths, the Genebank is well positioned to deepen its contribution to the conservation of agricultural biodiversity and support long-term food security.