



## **AGENT Project – Genebank Review**

Genebank Reviewed: Centre for Genetic Resources (CGN), Wageningen, The Netherlands

Date: July 19-20, 2022

Participants CGN: Theo van Hintum and other staff members

Reviewers: Katya Uzundzhaliyeva & Gergana Desheva (IPGR, Bulgaria), Isaura Martin & Luis Guasch (CRF, Spain)

### **Background**

Within the AGENT H2020 project, a new approach is tested to review the operations of European genebanks (GB) and guide their improvement through a system of reciprocal visits and support. The blueprint of a GB monitoring system, as adopted by the European Genebank Integrated System (AEGIS), will be tested by focusing on the European collection holders of wheat and barley cooperating within AGENT. This will serve as an example for wider use within the European network.

Curators of 11 GBs will visit each other's facilities and evaluate the efficiency of operations based on jointly prepared protocols. Reports will offer recommendations for improvement and will be used to approach suitable funding agencies for targeted capacity building. In the first cycle the genebanks of CRI (Czech Republic), NPPC (Slovakia) and IPK (Germany) are involved, in the second INIA (Spain), IPGR (Bulgaria) and WR (The Netherlands). This report reports on the second review in the second cycle: the Centre for Genetic Resources (CGN) of the Wageningen University and Research (WUR), The Netherlands.

### **Visit/Organization**

The second visit in the second cycle was organized by Theo van Hintum, head of the CGN plant genebank. He also provided an updated version of the CGN Operation Manual, already available online on ECPGR site, as well as statistical information about the genebank. The Manual gave the reviewers an excellent opportunity to prepare for the visit as it describes the organization and procedures of the genebank. Accommodation and flights were arranged by the reviewers and the genebank staff arranged transport from and to the hotel, as well as all the details to facilitate an optimal review work.

Based on an agenda for the two-day review, all aspects of the genebank could be reviewed and discussed. Theo van Hintum gave a comprehensive presentation of the CGN organization and procedures, including a short video connection with the rest of the staff and an overview of phytosanitary issues by an expert in this field. The reviewers visited the cereal and legume regeneration field, the seed desiccation and storage chambers, and the viability testing and packaging facilities, having the opportunity to talk with the staff involved. Greenhouses and plant

processing facilities of UNIFARM, the company that gives service to the WUR campus, were also visited, including specific seed cleaning and seed pre-storage equipments. During presentations and visits, extensive discussions were held on the topics covered. The CGN operation Manual was also reviewed to obtain further information or to clarify some aspects when necessary. At the end of the second day, the reviewers discussed and presented a first draft of their impressions of the genebank.

CGN as promotor of these kind of peer reviews is perfectly conscious of the benefits of this activity as a learning and improving experience. This understanding has been fully conveyed to the reviewers.

### **Outcome of the Review**

The first outcome of the review was the exchange of different approaches to conserve and use the PGRFA. Thus, from the very beginning, the presentation of the CGN gave the panel the impression that the genebank activities are very pragmatical and user driven, with a very intensive cooperation with the industrial sector. Its experience can help other genebanks to implement public-private partnerships.

Due to the composition of the collections, with a relatively small proportion of landraces, the CGN is not as focused on strict preservation of genetic material as other genebanks in which the proportion of endangered local material is much higher.

In addition to this general outcome, a number of observations were made, some of which can be translated into recommendations.

### Management/Funding

The Centre for Genetic Resources – the Netherlands (CGN) is located at Wageningen University and Research (WUR), The activities of this Centre on plant genetic resources (PGR) conservation started in 1985 from scientific working collections. Animal genetic resources (AnGR) were merged in 1999 and forest genetic resources (FGR) in 2022. In that period, CGN was managed as a state organization, and since 2004 it became a foundation that is operating under 5 year agreement with the Dutch Ministry of Agriculture, Nature and Food Quality (LNV) based on “statutory tasks”. At that point they were required to use ISO 9001 as internal quality management system. Thus, they became the first genebank in EU following an internal quality system, leading such initiatives internationally. Statutory tasks are funded by the Ministry LNV and CGN-PGR also receives funds from other sources (EU projects, companies, etc). Recently the available budget has increased substantially.

CGN genebank conserves over 23,000 accessions of 31 crops and is mainly focused on vegetables. CGN policy has always been to keep the collections within a manageable size. However, in agreement with the Ministry LNV and the production sector, it is now intended to follow a strategy of increasing the size of the collections, especially for crop wild relatives (CWR). This will require an increase of the space and facilities in the genebank.

### **Recommendation 1**

Preserve CGN leadership in promoting the implementation of quality systems, to help other genebanks to fulfil at least the minimum standards.

## Recommendation 2

Build larger facilities for long term conservation as well as viability control facilities accordingly

### Germplasm Management

The figures presented by CGN show that current management procedures are working pretty well so far. The way of processing the material in separated bags is oriented to be a practical method with a single procedure of conservation for all purposes (distribution, long-term preservation, viability control or black box). All viability testing are based on the premise that the seed are conserved under the same conditions reducing the need for double checks. User distribution bags that were previously stored at +4°C has been rearranged into individual freezers to have all the samples at -20°C.

As all the bags are stored under vacuum it is possible to detect any leakage or failure of tightness.

In fact, during the visit to the genebank facilities, the reviewers were able to detect vacuum leaks in some bags that were going to be used for germination tests. It seems that vacuum failures occur with certain frequency and are annotated on the corresponding germination sheets. However, so far no negative effects have been observed on seed germinability of these bags.

Vacuum losses can represent a major risk for long-term storage, as this may lead to an increase in seed moisture content, especially when it can take long time to detect failed bags.

## Recommendation 3

Review all the steps of sealing the bags including materials, procedures, time or temperatures of sealing, pressures or other parameters relevant for this key activity. Assess the incidence of the problem of bag vacuum losses. Evaluate the proportion of bags with vacuum failures after different period of time.

## Recommendation 4

Whenever possible, perform water activity measurements to check the seed moisture level (e.g. with a Rotronic equipment), especially for bags with vacuum losses, to detect a possible increase on seed moisture content. However, the minimum quantity of material required for these evaluations may be a limitation, especially in small seeds.

### Germination Testing

Currently, all germination tests are performed by CGN staff. After germination testing seed lots are scored from 1 to 4: (1) very healthy and well above the germination percentage threshold, (2) above the threshold, but showing signs of aging, (3) just on or below the threshold and in need of regeneration but not very urgently, (4) poor germination below threshold and in need of urgent regeneration. This is a very practical approach that facilitates the assesment of regeneration requirements and viability monitoring intervals, although it implies a certain degree of subjetivity.

The initial viability of every sample is checked before storage, retested after 25 years and then in 15, 10 or 5 years depending on the lot score (1, 2 or 3, respectively).

The panel discussed the general rule of setting the first viability monitoring after 25 year. This period seems too long, especially for species with seeds of low longevity. As these rules were set on the basis of previous data that were not very reliable and a new analysis is ongoing, it might be advisable to consider the different sensitiveness of crops to seed ageing, also taking into account that germination thresholds used (80%) are not the most restrictive - 85% is recommended in FAO Genebank Standards-.

As CGN is planning to grow rapidly the number of accessions, the seed viability monitoring capacity has to be increased accordingly. At this moment cereals testing is performed into greenhouses because the light of the genebank chambers is not enough to properly detect chlorosis as ageing symptom. CGN genebank should at least double the number of growth chambers to implement the new requirements.

Seed dormancy is a frequent phenomenon in wild plants such as CWR. When effective treatments applied to break seed dormancy are not available, germination values cannot be used as the only criteria for determining regeneration requirements or monitoring intervals. Other procedures, such as seed cutting should be employed to identify viable dormant seeds. In addition, X-ray images can be a very useful support to determine empty, damaged or immature seeds.

#### **Recommendation 5**

Review the period for the first viability monitoring accordingly to new data, considering the different sensitiveness of the species.

#### **Recommendation 6**

Increase the number of the germination chambers. Include control of humidity and light intensity

#### **Recommendation 7**

Especially for wild material: use special criteria for assessing seed viability. Use X-ray images to determine the proportion of empty/damaged/immature seeds.

#### Regeneration

The breeding sector feels that they should contribute to PGR conservation, so they are involved in regeneration activities of the CGN genebank. This is particularly important for allogamous plants. Health inspections are carried out by Plant Protection authorities in both companies and CGN regenerations.

#### Documentation

The documentation system used by CNG is very effective and adapted to their own procedures, however CGN complains about its cost and adaptation to other common systems (e.g. GRIN-Global).

Especially, the characterization/evaluation information system is impressive, friendly and oriented to users. All the descriptors are standardized what means a lot of work, but the result for the user is simple, very reliable and visible. Besides, you can get the original data without manipulation by downloading an excel file.

### **Final conclusion**

CGN constitutes a reference genebank especially for vegetable crops. The practical vision of the genebank management and the strong commitment with breeding sector is an example for the genebank community. Their will to undertake new collecting missions of CWR will increase their number of accessions, which will require more space and flexibility to deal with wild species. The problems detected on vacuum-sealed bags must be deeply investigated to determine the causes and to overcome this problem, including the adaptation of protocols.

### **Final remarks**

The reviewers were impressed by the excellent preparation, positive atmosphere and complete transparency presented by the hosts. As a result, the discussions were open and fruitful. This was very much appreciated.

July 25<sup>th</sup>, 2022

The reviewers: Katya Uzundzhalieva, Gergana Desheva, Isaura Martin, Luis Guasch