Ex-situ conservation of PGR in the National seed genebank of Bulgaria - past, present and future

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Abstract
The *ex situ* conservation of plant genetic resources in seed genebank plays an important role for food security in the future. The National Genebank of Bulgaria is located on the territory of the Institute of Plant Genetic Resources "K. Malkov" - Sadovo. It was built in 1984 and carry out a scientific programme for long-term preservation of germplasm with seeds under controlled conditions in accordance with the standards, developed by FAO. The total number of registered accessions in the Genebank is above 60 000, where 44572 are preserved in the base collection. These accessions are representatives of 32 families, 150 genera, 600 plant species. Total 10 193 of the accessions originated from Bulgaria. The accessions received abroad originated from more than 80 countries. Germplasm material consists of: local population, old cultivars, advanced varieties, breeding lines. The genetic stocks are available for research and for plant breeding, further utilisation by international and regional co-operation, free exchange of plant genetic resources according to CBD and conditions contained in the standard material transfer agreement.

Key words: *ex- situ* conservation; documentation; genebank; maintain; PGR

INTRODUCTION

The *ex situ* conservation of plant genetic resources for food and agriculture (PGRFA) is a global concern and plays a central role for food security in the future. It can be achieved through different methods such as seed banks, field genebanks, in vitro storage methods, pollen banks and DNA banks (Dotlačil et al., 2008; Dulloo et al., 2010; Krasteva et al., 2012; Börner et al., 2014). According to the Convention on Biological Diversity (CBD, 1992), it is a national responsibility of each country to ensure the rational conservation and sustainable use of plant genetic resources. This responsibility is usually delegated to a national genebank or run by programmes which involve collaboration with other public sector institutions and relevant partners within the country. The national genebanks have frequently been the leaders in driving the establishment of broader national genetic resources programmes, mainly by responding to the need for linkages between conservation and user communities of specific crops. National programmes and regional networks are widely considered to be the appropriate platform for implementation of the relevant international agreements such as the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (GPA, 1996, 2011), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, 2009) and the relevant objectives of the CBD (1992).

The Second report on the *State of the World's Plant Genetic Resources for Food and Agriculture* published by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2010) mention that there are about 7.4 million accessions conserved in over 1750 genebanks around the world in either seed banks, field collections, and *in vitro*

and cryopreservation conditions (FAO, 2010). Of these, more than 500 gene banks and other germplasm collections are situated in Europe, with combined holdings of about 2 million accessions (FAO WIEWS). The purpose of these collections is long-term conservation and to ensure the availability of plant genetic resources for present and future generations.

In Bulgaria the IPGR-Sadovo is the National Coordinator of the National Programme for Plant Genetic Resources as a part of the European Programme for Plant Genetic Resources (ECPGR). On the territory of the Institute is located the National Seed Genebank. The main mission of the National Seed Gene Bank is the conservation of plant genetic resources important for food and agriculture (PGR), their availability and distribution to local and foreign users in accordance with the ITPGRFA and Standard Material Transfer Agreement (SMTA).

The aim of this review is to present the role and importance of the National Seed Genebank in *ex situ* conservation of plant genetic resources in Bulgaria.

MATERIAL AND METHODS

As material 44572 seed accessions from the base collection of National Genebank of Bulgaria are used. The information for collection status is according to Multi Crop Passport Descriptors as international standard for EURISCO.

RESULTS AND DISCUSSION

History

The National Gene Bank was built in 1984 and carry out a scientific programme for long-term preservation of germplasm with seeds under controlled conditions in accordance with the standards, developed by FAO (1980/1994). The activity of the National Genebank following a sequence of events according to stages of development of the Program of Plant Genetic Resources (Stoyanova, 2003, 2007):

✓ **1977-1980** was revised the status of old seed collection moved from the Institute of Genetics (BAS). Laboratory tests were carried out of 80631 seed accessions, where 31149 were determined to be viable. As result of field regeneration were restored only 11118 accessions which formed the initial base collection of the National Genebank;

✓ **1980-1984** was developed seed technology for long-term storage according to the recommended genebank standards;

✓ **1985** -Opening the National Genebank. Started genebank samples supply under long-term and medium-term storage conditions;

✓ **1986-1996** Further development of specific approaches for different plant species in relation to their seed storability;

✓ **1997-2001** Creation of useful models for seed viability monitoring and for prediction of control tests frequency and regeneration needs;

✓ **2002-2007** Creation of contemporary electronic database by Multi Crop Passport Descriptors. Including in the European catalog for plant genetic resources (EURISCO);

✓ **2008-2009** Expand utilization of germplasm according with CBD, ITPGRFA and Standard Material Transfer Agreement (SMTA);

✓ **2009-2013** Participating in establishment of European Genebank Integrated System –*AEGIS*;

✓ **2014** Safety duplication of 933 accessions from National Genebank of Bulgaria to Svalbard Global Seed Vault;

✓ **215-2022** Preparation of operational genebank manual for The AEGIS Quality System (AQUAS);

✓ **2022** Establishment of Joint Chinese-Bulgarian Laboratory for Molecular Biology of Crop Germplasm Resources.

The National Genebank Collection – structure and maintenance

The genebank facilities are designed both for long-term storage and for medium-term storage. There are maintained three collections: base collection, working collection and collection for free exchange.

The base collection is for long-term conservation where the seeds are stored at 3–7% moisture (depending upon species) and at subzero temperatures (−18 ºC) in hermetically closed containers (glass jars or three laminated aluminum foil packets). Under these conditions the seed germplasm could be preserved with minimal changes over decades or hundred and more years. On the basis of control tests is determined the safety storage time for most of the preserved in the genebank plant species (Stoy-

anova, 2005, 2007; Desheva, 2016). The safety storage time of existing plant species varies from 20.41 years (*Arachis hypogaea* L.) to 500 years (for *Avena sativa* L. and *Triticum aestivum* L).

The working collection is for short-term conservation where seeds are stored at 6-7 ºC, air relative humidity 40-45%, and free access of air (paper bags). Under these conditions the mean seed longevity varies between 2 and 10 years. These collections are used for multiplication, distribution and characterization/evaluation.

The collection for free exchange is under medium-term storage condition, where seeds are stored at +6ºC but in hermetically closed glass jars.

Status of the base collection

The structure of base collection is presented in six groups (Fig. 1):

- Cereals – 26 568 accessions;
- Legumes – 6 698 accessions;
- Forage grasses – 1 432 accessions;
- Vegetables – 4 118 accessions;
- Oil and industrial crops – 3 455 accessions;
- Ornamental and medicinal species – 428 accessions.

These accessions are representatived from 32 families, 150 genera and 600 plant species.

Total 10 193 of the accessions originated from Bulgaria. From them 4 472 accessions are local populations and 4 850 are breeding materials (primitive varieties, breeding lines or modern varieties) and 871 accessions are with unclear biological status. The accessions received abroad originated from more than 80 countries.

Described by continents the pattern of origin is as follow: Europe – 22 489 accessions; Asia – 3253 accessions; North and South America – 3 165 accessions from 20 countries; Africa – 480 accessions from 7 countries; Australia – 113 accessions from 3 countries and Unknown origin – 15 073 accessions.

Genebank operations

Following receipt at the genebank, the seed samples are registered and added to the collection if they meet the minimum standards for germination, seed quantity and accompanying passport information. The operational sequence to integrate an accession into the genebank involves cleaning, moisture determination, drying, viability testing, quantity measurement and packing (Elis et al., 1985). The management of seed collections requires that germplasm accessions be maintained with a high proportion of viable seeds. This involves storage under optimal conditions, periodic monitoring of seeds for viability and quantity, and regenerating them when the situation warrants. The success of long-term conservation of seeds is dependent on continuous viability monitoring and regeneration or re-collection when the viability of the sample drops below a minimum level.

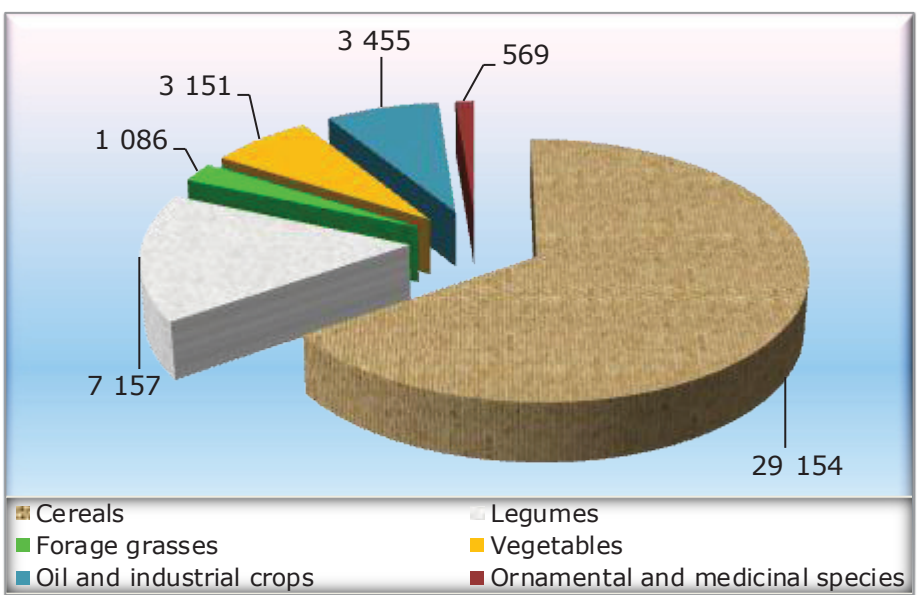

Figure 1. Status of the base collection maintains in the National Seed Genebank of Bulgaria

In the National Genebank of Bulgaria seed accessions in long-term storage are monitored 10 years after the start of storage. In cases where initial seed germination is poor because some species are not able to produce high quality seeds, then seed viability monitoring is carried out after 5 years of storage. The decision to regenerate germplasm accessions depends equally on the viability and quantity of seeds held in store. We are planned to regenerate an accession when seed viability fall above 10 % of initial viability or when number of seeds fall below 1000. Regeneration of seed germplasm is carried out in the experimental fields of the IPGR from curators of the Department Plant Genetic Resources.

Utilization of PGR

According to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), Standard Material Transfer Agreement (SMTA) and the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture, The National genebank of Bulgaria is partner in the system for free exchange. We meet the obligations of the Convention on Biological Diversity to facilitate information exchange and material transfer regarding the conservation and sustainable use of biological diversity that contributes to the Pan-European Biological and Landscape Diversity Strategy.

The exchange collection contains 2 421 accessions from 56 genera and 104 plant species.

During 2001-2021 the total number of accessions placed at disposal amount to 20 259, where: 12 382 accessions were sent to abroad and 7 872 accessions were given to Bulgarian institutions (Fig. 2).

Safety duplication

An important aspect of Bulgarian National genebank is to secure duplicates of germplasm for safety backup to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes. Therefore total of 933 accessions from basic collection of the National genebank of Bulgaria are maintained as safety duplicates in the Svalbard Global Seed Vault. The all materials from 14 crops (bread wheat-220, durum wheat- 207, barley-147, maize- 117, sorghum-97, rye-13, oats- 5, bean-37, chickpea-27, lens-20, faba bean-17, grasspea-10, cowpea - 9, and lettuce- 11) are unique local accessions (Fig.3). The most of accessions that are duplicated in Svalbard were regenerated by Global Crop Diversity Trust project: "Regeneration and Safety Duplication of Regionally Prioritized Crop Collections from Bulgaria" in the period 2008-2010. In the future National genebank planning to send accessions from other plant crops.

Documentation

Documentation is essential in good genebank management to allow efficient and effective use of

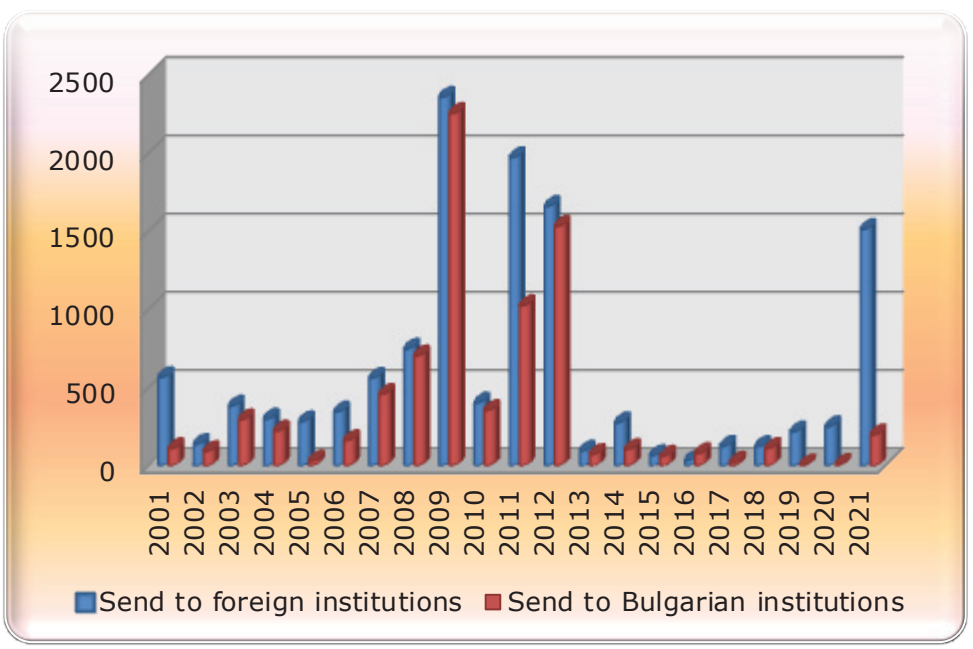

Figure 2. Number of accessions sent to different addresses in the world from 2001 to 2021

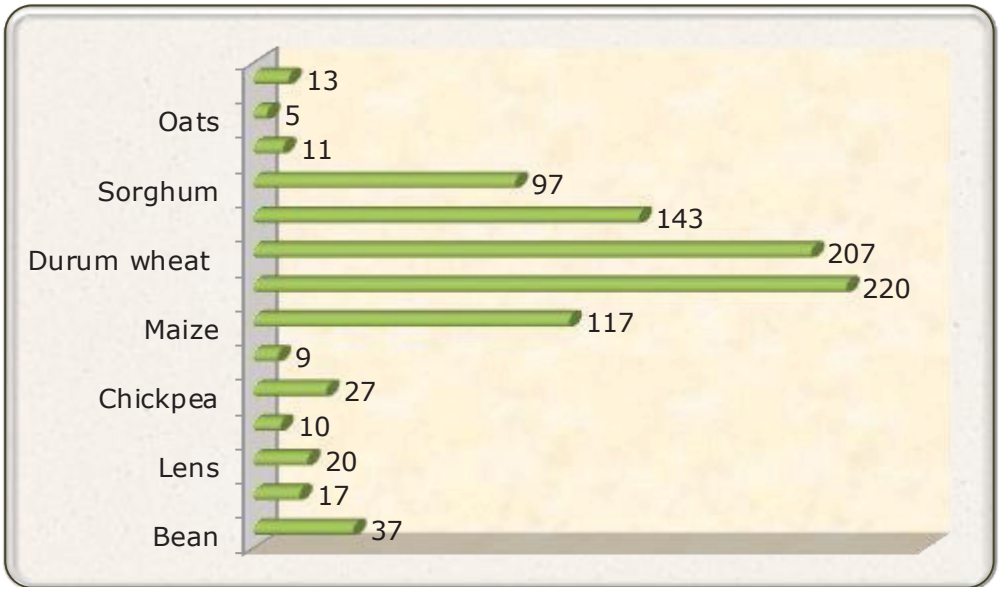

Figure 3. Status of the Bulgarian collection maintains in Svalbard Global Seed Vault as safety duplication

germplasm. Seed storage database allows control and monitoring of seed accessions preserved in the base collection under long-term storage conditions. All accessions are designed with BGR-number for identification in the National genebank. There are included some fields associated with passport data as well storage data (Stoyanova, 2002). Seed storage database is maintained in ACCESS format.

PGR management includes data for characterization, evaluation and storage. Evaluation data files are completed by curators of collections according to the recommendations of the crop working groups.

International cooperation

The international cooperation is one of the crucial activities of the work with plant genetic resources. The European Cooperative Programme for Plant Genetic Resources (ECPGR) plays importance role in ensuring the long-term conservation and facilitating the increased utilization of PGR in Europe. The Institute of Plant genetic resources joined the programme in 1983 and have been taking an active part since then. In 2001, the need for a European plant genetic resource information system was met with the development of national inventories of plant genetic resources and the creation of EURISCO, a searchable catalogue of *ex situ* plant collections maintained in Europe. The national gene bank in IPGR - Sadovo was nominated by the European PGR programme as a focal point for Bulgaria, which is a significant achievement for the agricultural science. Thus, the right of other institutes in the country to join the system and participate in the electronic catalogue is protected. Now passport data from 69 767 accessions are available in EURISCO as plant genetic resources in the Bulgarian National inventory (65 347 of which h are in the IPGR - Sadovo, 3 857 are at DAI - G. Toshevo and 563 in IREMC – Kazanlak (https://eurisco.ipk-gatersleben.de/apex/eurisco_ws/r/eurisco/home).

In phase VIII (2009 – 2013) of the ECPGR activities was started the European Genebank integration project - AEGIS. The goal of AEGIS is to create 'a European gene bank integrated system' for PGRFA, aimed at conserving the genetically unique and important accessions for Europe and making them available for breeding and research. Its aim is also to strengthen the cooperation in Europe and integrate the capacities while ensuring the highest quality standards and guarantee the accessibility of genetic resources to their user The Institute of Plant genetic resources (in particular National Genebank of Bulgaria) is joined to the project in 2009 with signed Memorandum of Understanding (MoU) (http://www.ecpgr.cgiar.org/aegis/).

Priorities in the future research

- Support of free exchange system for utilization of plant germplasm in the world.

➢ Improvement of approaches for conservation methods, monitoring viability and regeneration strategies aiming at maintenance of genetic integrity of plant *genetic resources*.

➢ Filling the gaps in collections (passports, characterization and evaluation data).

➢ Creation of National Genebank information system combining passport, characterization and evaluation data.

➢ Enrichment of existing collections with local landraces and CWRs.

➢ Collaboration at national, regional and global scale for conservation of LRs and CWRs diversity.

➢ Strengthening of collaboration, networking and linkages among various stakeholders at both national and regional levels through pooling of resources and use of comparative advantages available in the various institutions and countries.

CONCLUSIONS

• In the National Genebank of Bulgaria are maintained 44 572 seed accessions from 32 families, 150 genera, and 600 plant species, under long-term storage conditions according to preferred Genebank standards.

• Total of 933 accessions from basic collection of the National genebank of Bulgaria are maintained as safe duplicates in the Svalbard Global Seed Vault.

• The monitoring of seed viability is carried out every 10 years and the information of accession availability is maintained in ACCESS-database.

• The passport information is accessible as a part of European electronic search catalog – EURISCO (https://eurisco.ipk-gatersleben.de/apex/eurisco_ws/r/eurisco/home).

• The collections provide the good opportunity of utilization based on existing large scale genetic diversity: wild species, local populations, primitive varieties, breeding materials and modern varieties with different origin.

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