

Plant Genetic Resources and Cryopreservation

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3-4 MAY, 2023 PRAGUE, CZECH REPUBLIC

Introduction

Georgia is situated at the strategically important crossroads where Europe meets Asia. The country has a unique and ancient cultural heritage, and is famed for its traditions of hospitality and cuisine.

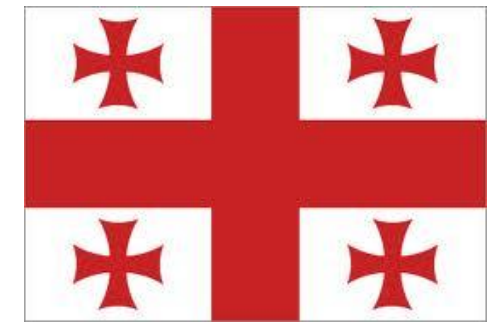
Georgia is a country in the Caucasus region of Eurasia. Located at the crossroads of Western Asia and Eastern Europe, it is bounded to the west by the Black Sea, to the north by Russia, to the south by Turkey and Armenia, and to the southeast by Azerbaijan. Georgia is also one of the first world countries that adopted Christianity as the state religion in the first half of the 4th century.

Capital - **Tbilisi**,

Population - 3.724 million

Area (in sq. km) 69.700

Language(s) Georgian



The State of Biodiversity

The Georgian flora is rich in economically important plant species.

According to N. Vavilov, Georgia is part of the west Asian center of origin of the cultural plants. West Asia is considered as a major center of domestication of barley, wheat, pea, lentil, vetch, grapevine and numerous fruit trees.

A variety of crops, such as cereals (wheat, barley, rye, sorghum, millet), legumes (faba bean, grass pea, chickpea, lentil, cowpea), also flax, onion, garlic, and various fruits (grape, apple, pear, quince, medlar, peach, apricot, plum, cherry, cornelian cherry, etc) have been cultivated here from ancient times.

Starting from the 17th century, many American crops were introduced such as maize, potato, tomato, tobacco in the country and which were followed by the citruses and tea in the 19th century and kiwifruit in 20th century.

State of the *in-situ* conservation

According to the Georgian Law of the System of Protected Areas, the following types of PA could be used for *in-situ* protection of PGR: state nature reserve, national park, monument of nature, protected landscape, multiple use territory and biosphere reserve. There are: 13 Natural reserve, 10 national park, 19 manage reserve, 40 natural movement, 2 protected landscape.



On-farm Management and conservation

Biological Farming Association “Elkana” works on the issues of the conservation of agricultural biodiversity since 1996. <http://www.elkana.org.ge>

Grape: local landraces, **Fruits :** (cherry, sweet cherry, Peach, Pomegranate, Apricot, Apple, Medlar, Hazelnut, Walnut, Strawberry, Blackberry, Cornelian cherry, Gooseberry, Currant, Quince, Fig, Pear, Almond, Raspberry, Cherry plum, Jujube, Plum), **Field crops: grasses:** Sainfoin, Alfalfa, ryegrass, Orchard grass, **Cereals:** Wheat, Triticale, Barley, Oat, Rye, **Legumes:** Pea, Common Bean, Chickpea, Lentil, Soybean, Common vetch, Faba bean, Cowpea, **Technical crops:** Peanut, Tobacco. Flax, **Millet crops:** Maize, Millet, **VEGETABLES :** Eggplant ,Pumpkin, Spinach, Cucumber, White-headed Cabbage, Melon, Celery, Garlic, Parsley, Tomato, Basil, Watermelon, Carrot, Chilli pepper, Table beetroot, Onion.



State of the *Ex situ* Management-Main *ex situ* Collections

Most of the plant research centers maintain *ex-situ* germplasm collections Georgia.

The largest collections of PGRFA have been concentrated at SCRA (Scientific-Research Center of Agriculture),

NBGG (National Botanical Garden of Georgia) is the largest centers of wild plant *ex situ* conservation.

- ✓ Scientific-Research Center of Agriculture (SRCA) presented with seed type and live collections;
- ✓ National Botanical Garden of Georgia (NBG)
- ✓ N.Ketskhoveli Institute of Botany (IB)
- ✓ Agrarian State University Gene bank

Scientific Research Center of Agriculture

<http://srca.gov.ge/en>

1. Fruit-growing Research Division
2. Research division of agro-forestry cultures
3. Research division of vegetable crops and melon cultures
4. Department of Integrated Plant Protection Research
5. Research division of microclonal (In vitro) propagation of plants and virus-free planting materials
6. Department of Livestock Breeding, Veterinary and Forage Production
7. Research Division of Viticulture and Wine-making
8. Division of Standardization, Certification, Planning and Economic Analysis, Filed crop seed Genebank
9. Research Division of Soil Fertility
10. Research division of cereal crops
11. Research division of tea and subtropical crops
12. Organic Farming division
13. Research Division of Agricultural Engineering
14. Risk assessment division
15. Research Division of Storing and Processing of Agricultural Products
16. Division of public, regional and international relations
17. Research division of ecophysiology
18. DNA Laboratory





Scientific Research Center of Agriculture

<http://srca.gov.ge/en>

Conservation Types: *Ex-situ* and *in vitro*

- Field Crop Seed Gene Bank –Cereal, Food legumes
- *Ex-situ* - Live collections of fruit crops, Grape collection, Agroforestry crops;

In vitro Conservation of :

- Potato (3 local , 10 introduced varieties);
- Clones from CIP (40 examples)
- Grape 30 varieties
- Wild grape 2 form
- Rootstocks 2 form



SRCA-Perennial crops genetic resources live collections

Place –Jigaura **TOTAL Acreage: 84.0 HA**

Collections: 41.2 Ha Reserved: 1,8 Ha

Fruit crops live collection : totally 413

Local Germplasm collection : Apple,Pear ,Cherry,Sour Cherry, Prunus cerasifera, Varieties:

- Local varieties and forms -152;
- Rootstock 16
- Introduced varieties- 275



SRCA-Grape genetic resources live collections

Grape - 927 accessions

Local varieties - 450

Wild grapevine - 120

Rootstocks - 11

Introduced - 424



SRCA-AgroForestry PGR

Plant collection

Live collections

Permanent collection of forest species - 528 Poplar plantations - 1460

Basic mulberry collection - 450

Mulberry motherblock- 352

Mulberry mother seed collection - 10

Database

www.forestgenresources.ge



Jigaura screen-houses

With support FAO was built two screen-houses

One for fruit crops and second for the grape to produce basic material

The management of the greenhouses complies with international standards for pre-multiplication centers.





SRCA-Seed Gene bank Main Activates:

Acquisition of new germplasm samples (sources – from collecting missions, local institutes, international gene banks)

Multiplication/Regeneration of germplasm

Characterization and preliminary evaluation

Documentation and exchange information on germplasm

Conservation of germplasm

Collaboration with other plant genetic resources centers

Organization of technical meetings and training workshops



Laboratory of PGR

Laboratory analysis of the quality of seed material of field crops.

Sampling, testing (purity, germination, weight of 1000 seeds) according to ISTA methods.

Determination of moisture content and test for viability.

SCRA-Seed GENE BANK

Priority given to –Endemic species , traditional landraces ,Local breeding varieties

The gene-bank characterizes and evaluates its material based on FAO and biodiversity descriptors and regenerates it

Main crops: Cereals, Food legumes,

Collection type -3 types

Working collection,

Active collection,

Basic collection

Conservation

Middle term 1-10⁰C ;

Long term -1-22⁰C

Type of conservation : Seed, Herbaria





SRCA Gene bank collection



More than 700 accessions of wheat collected at different times from different institutes and collecting missions are currently preserved in the Gene bank as a seed collection.

200 accessions of 8 varieties of beans, 5 varieties of corn;

The collections were created and preserved in accordance with the international Gene bank Standards for Plant Genetic Resources for Food and Agriculture



National Catalog of Agricultural Crops Permitted for Distribution on the Territory of Georgia

<https://gpvc.srca.gov.ge/catalogue/>

Nowadays at the national catalogue registered 5 traditional varieties of wheat and 57 local varieties of grape.



SCIENTIFIC-RESEARCH
CENTER OF AGRICULTURE



National Botanical Garden of Georgia

<http://www.nbgg.ge>



Ex-Situ Conservation

National Seed Gene bank

Wild flora seed gene bank
1843 accessions

cultural flora
155 accessions

Collections duplicates are kept at Kew (Kew Botanical Garden)

Ex situ Live collection

Demonstration field for cereal and legume crops at the territory of botanical garden

In situ field for the Georgian Almonds

(*Amygdalus georgica* Desf.)



Agrarian State University Seed Gene Bank :

Middle term conservation: cereal 573, millet 193, legumes 582, forage grasses 45, vegetable 157,

Long term conservation legumes 42 accessions

Grape live collection: local forms and varieties – 232; introduced – 10 varieties



State Legislation

The importance of protection and use of PGRFA has been reflected in the Georgia legislature since the 1960-s.

By the order of #59 in 1997, the President of Georgia emphasized conservation of PGRFA as a major responsibility of the agricultural research centers.

The Georgian Law on Grapevine and Wine of 1998 recognizes the PGR of grape as a major treasure of Georgia and commissions the government to support activities necessary for its conservation and use.

The Law on Protection of New Plant Varieties establishes framework for protection of intellectual rights of plant breeders and is compliant with the UPOV requirements.

In 2005, The Georgian government adopted the National Biodiversity Strategy and Action Plan, which emphasized importance of conservation of agro-biodiversity. It also emphasizes the need of developing a concept for protection and sustainable use of agrobiodiversity.

State Legislation

- ✓ Law of Georgia on Protected Areas
- ✓ Law of Georgia on Water
- ✓ Law of Georgia on Forest of Georgia
- ✓ Law of Georgia About Georgia's "Red List" and "Red Book"
- ✓ Law of Georgia On Allowing the Use of Agricultural Crop Varieties Subject to Mandatory Certification and Seed Production
- ✓ Law of Georgia on Grape and Wine
- ✓ The Law on Genetically Modified Living Organisms was adopted in September 2014
- ✓ A draft Law on Biodiversity is currently under consideration by stakeholders and will be harmonized with the Environmental Directives of the European Union.

International Agreements

Georgia signed the international CBD in 1994.

International treaty for PGR for food and agriculture was signed 2018

CBD related conventions /Cartagena Protocol in 2009.

Georgia is a member of the Commission on PGRFA and was among the countries that approved the GPA for Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture and the Leipzig Declaration in 1996.

Georgia was accepted to UPOV and it has developed UPOV-compliant legal framework for protection of plant varieties and variety release. Georgia is a member of WTO.

Georgia is signed up to the 7th phase (2004-2008) of “European Cooperative Programme for Crop Genetic Resources Networks - ECP/GR”. In 2018 Renewed membership of ECPGR

Nagoya protocol is not yet ratified. However draft Biodiversity Law considered by the parliament is based on the principles of the Nagoya protocol and the government considers to ratify it in the nearest future.

ECPGR and Georgia

Renewed collaboration and signed in 2019

Have members of the following crop groups: **Malus/Pyrus, Grain Legumes, Vitis, Wheat, Documentation and Information**

Country Quotas Phase X (2019-2023) – 6

Cryoprezervation in Georgia

- 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.
 - Georgia is rich in both wild and agricultural biodiversity of flora. Among them there are species that are important as genetic resources.
 - We have gene banks of plants in the country, but there are such important species whose seeds are difficult to preserve in gene banks, because they easily lose the ability to germinate. Cryopreservation is acceptable for their storage.

Future plans for cryopreservation

- ✓ Grapevine (*Vitis vinifera sativa* L.) is one of the most important crop in agricultural sector of Georgia.

- ✓ More than 1000 explants of autochthonous grape varieties, are now presented at scientific research center of agriculture, Jighaura collection. In addition of this, you can find 140 examples of wild vines (*Vitis vinifera sylvestris* L).
- ✓ Plants, which are nowadays presented at the collection, are described
- ✓ with international harmonized descriptors (UPOV, IPRGRI, OIV).
- ✓ Furthermore, DNA studies with DNA markers are conducted for these varieties to understand genetics better.
- ✓ one of the most important crop regarding cryopreservation is grapevine.
- ✓ For our research team, it is also interesting to study orthodox and recalcitrant seeds. Orthodox seed can survive during drying and freezing in ex-situ conservation, while recalcitrant is not able. Cryopreservation can be a good way to manage this problem.

Recent research activities

- The recent researches dated 2019-2021 belongs to Professor Mariam Gaidamashvili and Tamar Kuchava.
- The research was conducted on chestnut trees -Efficient Protocol for Improving the Development of Cryopreserved Embryonic Axes of Chestnut (*Castanea sativa* Mill.) by Encapsulation-Vitrification.
- Study highlights that, Chestnut (*Castanea sativa* Mill.) is the dominant of mountainous forests of Western Georgia. Chestnut in the Red List of Georgia IUCN category is represented by vulnerable status (VU).
- Research offers that ,one of the most important ways is its long-term conservation at ultra low temperature (-196 °C) in liquid nitrogen. Freeze-preservation is the only technique, which guarantees the possibility of the problematic species, such as non-orthodox seed, also vegetatively plants .
- As a result, an optimized cryopreservation protocol for embryonic axes (EAs) of chestnut (*Castanea sativa* Mill.) has been developed based on the encapsulation-vitrification procedure.
- Nowadays, no recent activities are presented regarding cryopreservation in Georgia in literature review.
- <https://pubmed.ncbi.nlm.nih.gov/33504018/>

Cryopreservation as a tool to help in virus elimination process

- Cryopreservation combined with tissue culture (in-vitro) propagation is a good way to eliminate viruses like GVA. (after cryopreservation the success of virus elimination was around 97%)
- For today, we are studying and analyzing viruses at the virology laboratory of scientific-research center.
- The detection processes are done with helping of PCR, RT-PCR and ELISA tools.
- SRCA uses thermotherapy for virus (leafroll, fanleaf...) eradication, which follow the international protocol (EPPO).
- Sometimes viruses are stronger and still survive after thermotherapy. In this case, one of the solution can be **cryopreservation/cryotherapy**.

Cryopreservation in clonal selection

- ✓ Scientific-research center aims to have successful clonal selection process, which follows the international protocol to ensure the success.
- ✓ After clonal selection, there is a chance to get new plants/clones, which are more resistant to abiotic or biotic stress. New or old elite clones should be conserved correctly to maintain their genetic line, to be sure that their distinctive genetic trait is conserved.
- ✓ As it is known, thermotherapy or in-vitro propagation have several influence on plant genetics. It means that these tools can alter genes.
- ✓ **Cryopreservation** can be a good tool to **avoid alteration in genetic stability**, which is one of the aim of our country.

Conservation for a longer period

- ✓ Collections are two types-active and base.
- ✓ In the case of active collection, everything happens in a small period of time, which includes plant propagation and delivery.
- ✓ It gives ability to conserve genetic material and clonal lines in the field, orchard, greenhouse or in-vitro gene-bank. Contrary, base collections are saved for a longer period.
- ✓ It is important ensure the genetic stability of preserved material and its ability to germinate again.
- ✓ In-vitro conservation, ex-situ or in-situ collections are not the safest way to conserve plants. But, in addition of cryopreservation, all of them can be back-ups for each other. It helps our country to be sure that our genetic resources are safely stored for a long period of time.

Svalbard Global Seed Vault

Svalbard Global Seed vault-dome for seeds to be protected.

There are around 1700 gene banks presented around the world, but many factors can be dangerous for them.

Seed vault is a back-up for most of the countries, because it saves duplicates of different crops. It will be a great chance for our country to ensure our plant's safety at Svalbad.

Collaboration opportunities:

- ✓ Collaboration with different institutions and organizations will be helpful to **find** and **share** resources, to **make trainings** about cryopreservation and its specifics and have **new connection** with other colleagues.
- ✓ **Working hard** with each other can ensure our **success for future**.



Thanks for Attention