Conservation and management of plant genetic resources in the Insitute of plant genetic resources, Sadovo, Bulgaria*

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Abstract. The preservation of plant biodiversity of Bulgarian flora is the main priority in the scientific activities of IPGR – Sadovo that is a part of the National programme in Plant Genetic resources through realization of the "Conservation, Management and Use of PGR in Bulgaria" Project. The main goal is the conservation of the national plant biodiversity. Modern agriculture is based on a limited range of varieties and a few species. Generations before us have used countless local forms with large genetic variation, even within one country and region. The conservation and use of old plant material provides researchers, now and in the future, with valuable germplasm resistant to biotic and abiotic factors, many of which are stored only in the gene bank of IPGR – Sadovo.

Key words: *ex situ, in situ, in vitro*, genetic, plant, resources, storage

Introduction

During the recent decades the importance of biodiversity conservation for ensuring the food security of humankind has been well recognized. According to the Convention on Biological Diversity (CBD) sustainable use and conservation of crop diversity is a national task, duty and responsibility of each country. The preservation of plant biodiversity of the Bulgarian flora is a major priority in the scientific activity of IPGR – Sadovo. That is a part of the National program in Plant Genetic resources (PGR) through realization of the project: "Conservation, management and use of PGR in Bulgaria". The main goal of the project is conservation of the national plant biodiversity.

Bulgaria is a counry with very rich plant biodiversity. Despite its small territory (110910 km^2), the country comprises 4 biogeographical regions – alpine, sea, continental and steppe. The variable topography, geology, specific microclimate conditions and millennia of human activity on the territory of the country determine the rich diversity of species, communities and habitats, most of which of conservation importance. The existence of this exceptional plant diversity on the territory of our country imposes to us the responsibility to identify the threats related to conservation. The most essential threats are caused by the climate changes, but not less destructive for biodiversity are human activities.

The negative influence on biodiversity in agricultural lands is due to two basic reasons:

- ✓ The current agricultural policies, stimulating the intensive agricultural production at the expense of the biodiversity;
- ✓ Abandonment of rural areas with specific infrastructure constraints, limited employment and demographic changes there.

Even back in 1976, the International Food and Agriculture Organization (FAO) found that "genetic resources, thanks to which we live, are rapidly depleting"

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and require collection and storage in living collections (FAO 1976).

Adoption of several laws and documents on the conservation and wise use of plant genetic resources (Biodiversity Act, Medicinal Plants Act, the National Development Plan for organic farming, Natura'2000) is dictated by several preconditions:

- ✓ The existence of significant plant diversity in Bulgarian flora and many old local varieties, populations, ecotypes.
- ✓ The complex character of the biological ponetial of the local PGR, defined by the values they posess (agrobiological, genetic, ecological and economic) determined prior to their high ecological plasticity, adaptability and stress resistance to biotic and abiotic factors.
- ✓ The increasing threats for the genetic diversity due to climate changes, human activity and insufficient concern by the national authorities;
- ✓ Need for structural changes in agriculture and the challenges of new forms of land use, their closer integration to maintenance and preservation of ecological balance in agricultural ecosystems.
- ✓ Educational programs for increasing the public awareness on biodiversity and the formation of adequate environmental culture.

The purpose of this paper is to present the activities of IPGR – Sadovo on conservation and management of plant genetic resources in Bulgaria.

Results and discussion

The Institute for Plant Genetic Resources (IPGR) is the national coordinator for the conservation of the diversity of cultivated plants and their wild relatives within the European program for plant genetic resources – ECPGR. The scientific program is preapared in accordance with the priorities of the European Commission for Agriculture and Rural Development (Anonymous 2013).

In IPGR is situated the National genebank, which guarantees the preservation of seed material under conditions for long term storage. The collections that are stored in the National gene bank contains over 59000 seed samples from 1544 plant species. Over 70 % of the samples are placed in conditions for long term storage. Preservation of the original germplasm includes not only the products of plant breeding, but also traditional for the country but still little known materials:

- ✓ medical, ornamental, oil and spice plant species;
- ✓ "abandoned" species, which can be used in bio-agriculture (leaf vegetables, roots and nuts);
- ✓ local varieties, tradition for Bulgarian kitchen.

The activities of the Programme of Plant Genetic Resources in IPGR are in several directions: collection, assessment, storage and use of original samples originated from the country and abroad.

Priority topics in the work program on the PGR are **enrichment of the collections** with geneplasm with different origin and type of material; expand knowledge on the biology, interactions and reactions of ecotypes and environment. Enrichment of the collections with new plant germpalsm is done by free exchange with foreign genebanks and botanical gardens and by expeditions in Bulgaria and abroad in the frame of different projects. In recent years collecting is focused on local varieties and populations, wild species, crop wild relatives, rare, endangered and endemic species and varieties. Every year the collections are enriched with 500-1000 new specimens (Guteva & al. 2009; Guteva & al. 2011; Petrova & Angelova 2011a, 2011b; Petrova & Angelova 2013).

IPGR posesses the richest collection of species and varieties structured into six groups: grain cereals, pulses, fodder, technical, vegetable, medicinal and ornamental.

Of great significance for geneplasm usage is the complex character of **the assessment of the accessions in the *ex situ* collections**. That assessment is done according to the international descriptors of UPOV and methodologies consistent with European standards, which makes the results comparable to the genetic diversity of everyother country that is potentially usable.

As a result of the complex study of PGR in cereals (common wheat, barley, durum wheat, oats, rye, maize) are selected large number of samples with outstanding economic performance in grain yield, early ripening, cold tolerance, drought tolerance. These samples are subject to biochemical analysis and phytopathological evaluation as well (Chamurliyski & al. 2012; Chipilski & al. 2012a, b; Kachakova & Desheva 2013; Desheva & Kachakova 2013; Antonova & al. 2013; Popova & al. 2013; Pencheva & al. 2013).

In the group of grain legumes are established accessions with high winter resistance, early ripening, high productivity, resistance to lodging, high crude protein content, etc. In the group of forage grasses are selected samples with proven high yield of green and dry mass (Sabeva & al. 2011; Stoilova 2011; Petrova & Angelova 2011a, b; 2013; Guteva & al. 2011; Berova & al. 2012; Petrova & Mangova 2013; Petrova & Stamatov 2013).

In the group of oil, ornamental and medicinal plants accessions are evaluated according to productivity, ampleness and duration of flowering (Uzundzhalieva 2011; Stoilova & al. 2012).

Through research on the resistance of plant genetic resources to economically important diseases and pests are selected a large number of samples from different cultivars that carry valuable economic qualities and possess complex resistance to various pathogens of viral, bacterial and fungal origin (Chavdarov & Petrova 2011, 2013; Petrova & Chavdarov 2011; Chavdarov 2012, 2013; Chavdarov & Stoilova 2013; Desheva & al. 2015).

Conservation of the crops and their wild relatives is done through *ex situ, in vivo* (in the Botanical garde), *in vitro* and *in situ* methods.

Ex situ conservation in seed genebank

Bulgaria is a party to the Convention on biological, as well as partner in the European biodiversity program. The storage activity of plant diversity of crops is part of the National Action Plan for Biodiversity Conservation. With the creation of the European electronic catalog *EURISCO* in 2003 (http://eurisco.ecpgr.org) it has become clear that IPGR has the richest collection of plant germplasm stored in genebank in Southeast Europe.

The collection maintained in the National Genebank in medium and long-term storage conditions comprises 59 187 seed samples, 13 269 of which are with Bulgarian origin, 238 cultivated types: 33 cereals, 34 grain legumes; 41 for technical use, 29 vegetables, 43 forage, 19 ornamental. Priority is given to the research and storage of local Bulgarian geneplasm. With largest number of local accessions is the collection of cereals – 2916, followed by vegetable crops – 2154 and legumes – 410.

The conservation of seeds in the National genebank is done in accordance with the established international standards for genebank conservation (Anonymus 1994; Engels & Visser 2003; Rao & al. 2006). Thus safety conservation of the seeds from 1400 plant genera for 18 to 152 years is ensured (Stoyanova & al. 2009; Desheva 2009, 2010, 2012). Preservation of identity of plant germpalsm is done by limitation of genetic erosion and loss of Bulgarian resources. The control of the genetic identity of the accessions in the National genebank is done by application of genetic markers according to the methods of ISTA and UPOV (Stoyanova 2002; Stoyanova & al. 2011). Thus heterogeneity of the accessions at molecular level is determined, duplicates are established and the control of species identity in reproduction is ensured. As a result from the investigation of wheat varieties it is established that the variety heterogenity determined by genetic markers according to the methods of ISTA comply with the level of variation of the quantitative characters. These characters to some extent are influenced by the environmental factors, so in arbitration for identity it is necessary to conduct a parallel control by biochemical genetic markers. Database for identity of the genotypes according to gliadin specters is done and their identity/variation is evaluated using cluster analysis (Desheva 2009). The risk for multitime regeneration of the heterogenic accessions is proven, as a aresult of upredictable identity changes due to influence of environmental factors on the productivity of the single genotype (Desheva 2009).

A pattern monitoring by "filter endangered specimens" prove the possibility of anticipating the need for regeneration of endangered specimens. This is the guarantee of predictability of the results of storage in gene banks for an extended period of time. Success in storage of original germplasm by seeds in the National genebank is a ground for scientists from other institutes from Agricultural Academy and the Bulgarian Academy of Sciences to provide their varieties and lines, as well as seeds of rare and endangered species for long term storage.

In vivo conservation

The Botanical garden is a specialized unit of the Program of Plant Genetic Resources of IPGR – Sadovo. It was established in 2002 with the main goal for the preservation of local resources through *in vivo* and *in garden* conservation. There are kept rare, endemic, and endangered plants belonging to 54 families. From them 8 are rare Balkan endemics – *Achillea cl-*

ypeolata, Allisoides bulgarica, Knautia macedonica, Chamaecitisus janke, Iris reichenbachii, Iris suaveolens, Aegilops cylindrica, Haberlea rhodopensis; 5 are Bulgraian endemics – *Allium rhodopaeum, Sedum album, Vicia incisa, Aegilops neglecta, Soldanella rhodopaea***;** 4 are endangered plants – *Leucoyum aestivum, Artemisia pedemontana, Anemone sylvestris, Pyracantha coccinea*; 11 rare plants – *Meum athamanticum, Artemisia lerchiana, Artemisia pontica, Leontopodium alpinum, Leucanthemum vilgare, Andrachne telephioides , Aegilops triuncialis , Koeleria brevis , Secale cereale var. perene, Clematis alpina, Paeonia tenuifolia.* The medical plant collection, kept in the botanical garden comprises 51 species. The specimens in the botanical Garden are divided thematically as follows: essential–oil plants; grasses; forage crops; ornamental plants; rare and endangered wild plants; crop wild relatives; introduced plant species.

Demonstrative collections in the Botanical garden are made of species that are crop wild relatives, old varieties and ecotypes: *Beta maritima, Trigonella coerulea, Luffa acutangula;* old pea varieties as well as introduced plant species like *Physalis peruviana, Cynara scolymus*, *Cynara cardunculus.*

In vitro conservation.

For conservation of vegetatively propagated plant species the most suitable method is *in vitro* conservation. Of most economic importance for our country are potatoes, vine, mint and hop. Technologies for *in vitro* micropropagation and conservation are created for them. As a result *in vitro* collections for continuous investigations from these species are created, including a significant, but not the full range of varieties from these species in the country. Completing the *in vitro* collection continues each year, after conducting preliminary tests for *in vitro* cultivation and micropropagation for each individual variety.

In the "Plant biotechnology" department has developed and applied different methods for long-term storage of specimens of those species by using low positive temperatures, growth inhibitors, osmotic stress, reducing the composition of the media and their combined application.

In vitro collection of *Vitis* spp. contains 103 varieties and rootstocks (Table. 1) that are maintained at low positive temperatures and by reduced composition of the culture medium.

Table 1. Varieties and rootstocks from *Vitis* spp. stored *in vitro*.

CULTIVARS			
Cabernet sauvignon	Chaush	Gamza	Evmolpia
Misket Hamburgski	Fetyaska alba	Slavyanka	Misket cherven
Bolgar	Korintsko cherno	Mavrud	M. sungurlarski
Rezekia	Armira	Pino gri	M. sandanski
Dimyat	Pino shardone	Uni byal	M. vrachanski
Pamid	Nemski risling	Misket Hamb. – cherven	Kamchia
Pino noar	Cherna perla	Zarchia	Aheloi
Rkaciteli	Перл de csaba	Shardone	Pizling bulgarski
Italia	Byalo bez seme	Buket	Nadejda
Merlo	Aligote	Hebros	Orfei
Super ran Bolgar	Shevka	Rubin	Pomoriiski biser
Brestovica	Chere pamid	Ranna meln. loza	Chernom. elecsir
Misket o'tonel	Pleven	Trakiiska slava	Chernom. brilyant
Kardinal	Shasla dore	Melnshki rubin	Biser
Dunav	Veren	Velika	Rusensko edro
Lubimec	Prista	Misket Rusenski	Misket plevenski
Misket Trakiiski	Palieri	Naslada	Drujba
Mechta	Parvenetz	Ticha	Sredetz
Kondaev 6	Rusalka	Rusalka 1	Rusalka 3
Misket Kailashki	Rizling italianski	Storgozia	Risling bulgarski
Ryahovo	Rusensko edro	Rusenska bez seme	Siyana
King Rubi	Kondarev 10	Kishmish Moldavski	Kishmish Luchisti
Vita	Maritza	Bezsemenen hybrid VI-4	Hibrid 6
Shiroka melnishka loza	Tamyanka	Kuklenski mavrud	Diana
Keratzuda	Senzo	Aligote	
ROOTSTOCKS			
Rupestris de Lot	Kobber 5BB	Ferkal	SO4

From Solanaceae *in vitro* are stored 170 varieties, from Bulgarian and foreign origin. Growth inhibitors are applied to secure long time storage and preservation of vitality at the same time (Table 2).

Table. 2. ***In vitro*** **collection of potato varieties.**

Sandra	Lizera	Romano	Sapfir	Aktiva
Montor	Borniya	Rekord	Sitovo – 2	Gala NN
Ponto	Andra N	Mariela	Vyatka	Djili
Kristi	Vila A	Elenite	Kastor	Pai kuin
Rila	№ 1257	Huron	Debora	Alegria
Oreb	№ 1723	Ormonte	Arenza	Audi
Picaso	Raya	Faust	Sviks	Vera
Almera	Agria	Element	Katadin	Folva
Lorh	Armada	Salut	Marita	Atlas
Stolovi	Agata	Burs	Mizafora	Atlantic
Dita	Artemis	Nimca	Zaicig	Bintdenks
Kleopatra	Impala A	Borko	Agro	Nordoltruzet
Aula	Riviera	Boeslam	Spekula	Triumf
Marania	Almera A	Moni	Shvalbe	Richi-Djudi
Trezor	Ariel	Final	Kosmos	Rusen-Barbi
Delkora	Konkorde	Rustika	Pioner	Rediontiak
Alina	Arinda A	Fezia	Bor	Sako
Kventik	NA 77154	Naidrum	Lola	Alfa
Lipsin	Azalia	Kibora	Elf	Adzimba
Amex	Sitovo – 1	Aksilia	Anushka	Esterlink
Umpala	Koreta – H	Dunia	Laidy Kler	Vokal
Sinora	Provolski	Falma	Santana	Akerseten
Arinda	Djiel 77156	Arnova	Triplo	Erik
Latonya	Vitorini	Kondor	Klarisa	Nait roz
Konkurent	Empere	Provento	Bel roz	Kristal
Ibor	Manna	Rojen	Sprint	Fortuna
Lincer	Daria	Perun	Kamila	Penobokat
Sokol	Provita	Izera	Solara	Trine kobler
Ropta	Ipmgard	Humalda	Ekstorit	Karmikavski
Firita	Atlantic	Agnes	Solist	Majestic
Viking	Eseks	Ontario	Pimpernel	Grantonkana
Sedvoya	Deirel	Banks ring	Burbang	Nofland
Dazos		Arlinnjio		Hindenburg

The storage conditions of 17 hops varieties, 13 varieties of mint and 18 medicinal species were tested and the results are shown in table. 3

Table. 3. ***In vitro*** **storage of hop, mint and medical plant species.**

Humulus lupilus **L.**		***Mentha*** **ssp.**		**Medicinal plants**	
Admiral	Lublin	M. aquatica L.	Mentolina 14	Stevia	Arnica
Bullion	Nugget	M. arvensis	Mentolina 18	Brosh	Gliciriza
Cascade	Opal	M. spicata	Mizia	Beladona	Mursalski chai
Chinook	Perle	M. piperita LL.	Lina	Ch. kantarion	Henopodium
Citra	S. Goldings	MPI	Zefir	J. kantarion	Hisop
Crystal	Target	MI	Mechta	Maiorana	Blatno kokiche
Galena	Taurus	Tundja	Nana	Nepeta	Merdjan
Herkules	Tradition	Mesten	Kliment	Salvia	Sladak koren
Ultra		Sofia		Matochina	Lavandula

In situ conservation

Result from the on-field studies are gathered from the typical localities of *Festuca pratensis* and *Festuca arundinacea* in meadows and pastures in different giogeographic regions. A database of the accessions and localities is established.

Localities of *Lathyrus cicera, Lathyrus sativum, Pisum elatius, Cicer monbretii, Vicia villosa* and *Vicia incise* are located and data for the habitat conditions are collected for *in situ* conservation.

Information about species from *Asteraceae*, *Brassicaceae*, *Apiaceae*, *Chenopodiaceae*, *Fabaceae*, *Lamiaceae*, *Rosaceae*, *Poaceae*, etc. are collected. These families consist of great number of crop wild relatives with valuable practical application (source of oil and other biological substances, ornamentals, vegetables, spices, etc.)

When there are many wild relatives and valuable PrP with versatile use, different floristic regions are investigated and the foundation for a dynamic storage (*in situ / on farm*) is established (Uzundzhalieva 2011). Unlike *ex situ* conservation, in which there is a long tradition, for the *in situ* conservation there is no common methodology and there is lack of coordination between institutions with responsibilities for conservation of biodiversity.

Documentation

In IPGR – Sadovo is supported the National register for plant genetic resources as a part of the European electronic cataloqie EURISCO (http://eurisco.ecpgr.org).

International cooperation

There is good coordination between IPGR as national coordinator for the PGR and the International Institute of Biodiversity (*Bioversity International*). IPGR is also a member of the *European Cooperative Programme for Plant Genetic Resources* (*ECPGR*). By signing in 2009 of the Memorandum of understanding for the establishment of a *European Genebank Integrated System – AEGIS* (MoU, 2009) IPGR participate in the establishment of the so called "Virtual" European gene bank, including European collections of "unique accessions" (http://aegis.cgiar.org). The main objective is to improve coordination concerning the protection of PGR in Europe and facilitate the exchange of PGR and related information. AEGIS database provides easier access of potential users to stored genetic resources in Europe, which in turn will lead to the elimination of restrictions associated with spatially remote location of a gene bank and as a result will improve coordination between organizations and researchers working with PGR.

The National gene bank was nominated by the European program for PGR as the focal point for Bulgaria. Thus is ensured the right to participate in the electronic catalog EURISCO.

Priorities for future activity in PGR Program in the IPGR – Sadovo:

- ✓ Close interaction with the scientific and educational centers in Bulgaria;
- ✓ Collection of the existing old varieties and populations of vegetables, grain legumes, cereals, forage, ornamental and other crops;
- ✓ Establishment of new territories and producers for *in situ* and on-farm conservation;
- ✓ Creation of demonstrative collections and trials with educational and practical purposes (with wild species, very old varieties, interesting for cultivation plants etc.);
- ✓ Creation of seed plots with wild forage, medical, oil and other groups of plant species for reintroduction or improvement of areas of high nature value.
- ✓ Preparation of a plan for interaction between biodiversity conservation and agriculture to establish good practices.

Modern agriculture is based on a limited range of varieties and a few species. Generations before us have used countless local forms with large genetic variation, even within one country and region. Conservation and use of old plant material gives the researchers, now and in the future, valuable germplasm resistant to biotic and abiotic factors, many of which are stored only in the gene bank of PGRI – Sadovo.

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