

## STATUS OF THE MEDICINAL AND AROMATIC PLANTS COLLECTION IN THE NATIONAL GENE BANK OF BULGARIA

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### Abstract

*Bulgaria is known for its wealth of medicinal plants. The diversity that exists ecologically is a prerequisite for the collection and cultivation of various species and forms with a high content of biologically active substances. The country therefore has centuries-old traditions relating to the collection, cultivation, processing and marketing of medicinal plants. More than 250 species are used in official medicine and are very well accepted in both the domestic and foreign markets. The collection of medicinal and aromatic plants in the National Genebank in IPGR-Sadovo, Bulgaria is represented totally by of 484 specimens of which 210 are annuals, 66 perennials, 26 bulbous flowers and 182 medicinal specimens, mainly wild.*

**Key words:** medicinal and aromatic plants, conservation, collection, PGR, National Genebank.

### INTRODUCTION

Medicinal and aromatic plants have been used by humans since ancient times. Nowadays the genetic diversity of medicinal and aromatic plant species in nature is under a threat of severe reduction or even extinction, due to the urbanization of the areas of natural habitats, to harvesting of raw material or to land use changing. The aim of this paper is to give overview for the status of the medicinal and aromatic plants collection in the Bulgarian genebank in IPGR-Sadovo as well as the methods for their conservation.

### MATERIALS AND METHODS

Bulgaria is situated in Southeast Europe, at the Balkan peninsula. The land area of Bulgaria is 110,994 square kilometres.

Despite the relatively small area, the relief of Bulgaria varies quite a lot. There are extensive lowlands, plains, hills, low and high mountains, many valleys and deep gorges. The main characteristic of Bulgaria's topography is four alternating bands of high and low terrain that extend east to west across the country. From north to south, those bands, called geomorphological regions, are the Danubian Plain, the Balkan Mountains, the Transitional region and the Rilo-Rhodope region. The easternmost sections near the Black Sea are

hilly, but they gradually gain height to the west until the westernmost part of the country is entirely high ground.

The specific geographical position of Bulgaria as the southern border of the Central European flora, the northern border of the Mediterranean flora and the western border of the East Asian flora determines both the rich diversity and the large number of endemic plant species. Of the 3567 known species of vascular plants, about 750 are medicinal. Most (85%) of the medicinal plants are wild and only about 15% have been introduced into culture and are cultivated. Many of the wild species are rare or protected (Popova & Marinkova, 1999; Jordanov, 1976).

The goal of conservation in the PGR is to ensure sustainable development by conserving and using biological resources in a way that does not reduce the world's genetic and species diversity and does not destroy habitats and ecosystems. It includes activities such as collection, propagation, characterization, assessment, conservation and distribution. The conservation of plant genetic resources has long been realised as an integral part of biodiversity conservation. There are two methods of conserving plant genetic resources - *in situ* and *ex situ* conservation. *In situ* conservation is the process of protecting plant species in their natural habitats. *Ex situ* conservation is the conservation of plant species outside their natural habitats. This type

of conservation is implemented through seed banks, field gene banks, in vitro conservation, pollen and DNA banks and cryopreservation (Engels & Visser 2003).

## RESULTS AND DISCUSSIONS

At the Institute of Plant Genetic Resources - Sadovo medicinal plants are stored in the National Genebank and in a living collection. The conservation of seeds of plant species under controlled conditions at the Sadovo Institute began in 1980. Currently, over 62 000 seed accessions are preserved in the National Genebank. Three types of collections are maintained - long-term storage, active and exchange collections. In long-term storage, seeds are stored at 3-7% moisture (depending on the species) and at -18°C in airtight three-layer aluminium foil packets. Active collections are for medium-term storage, where seeds are stored at 6-7°C and 40-45% relative humidity with free access to air (paper bags). In

the exchange collection, seeds are stored at 6-7°C (Rao et al., 2006).

The collection of medical species in the IPGR - Sadovo includes 80 genera, 28 families, and 173 species. The total number of stored specimens is 6398. The greatest species diversity occurs in the genera *Allium*, *Brassica* and *Lathyrus*, followed by *Centaurea*, *Helianthus*, *Impatiens* and *Vicia*. The remaining genera include between 1 and 3 species. The family *Asteraceae* is represented by 22 genera, *Brassicaceae* by 9, *Lamiaceae* by 8, *Fabaceae* by 7, *Apiaceae* by 6 and *Solanaceae* by 3, *Caryophyllaceae*, *Plumbaginaceae* and *Ranunculaceae* are composed of 2 genera, and the remaining 19 families are represented by a single genus. The medicinal plants of Bulgarian origin are 1059 specimens. They belong to 61 genera, respectively 21 families (Table 1). In the National Genbank are maintained 42 accessions included in the Medicinal Plants Act of Bulgaria (Table 2).

Table. 1. List of the medicinal plant species in the collection of the National Genebank in IPGR - Sadovo

Family	Genera	Number of species	Number of accessions	Number of accessions with origin from Bulgaria
<i>Amaryllidaceae</i>	<i>Allium</i>	17	291	75
<i>Apiaceae</i>	<i>Anethum</i>	1	5	3
<i>Apiaceae</i>	<i>Carum</i>	1	1	1
<i>Apiaceae</i>	<i>Coriandrum</i>	1	3	3
<i>Apiaceae</i>	<i>Foeniculum</i>	1	8	6
<i>Apiaceae</i>	<i>Opopanax</i>	1	1	1
<i>Apiaceae</i>	<i>Pimpinella</i>	1	10	2
<i>Asteraceae</i>	<i>Achillea</i>	1	1	1
<i>Asteraceae</i>	<i>Artemisia</i>	1	1	1
<i>Asteraceae</i>	<i>Calendula</i>	2	24	0
<i>Asteraceae</i>	<i>Carthamus</i>	1	14	0
<i>Asteraceae</i>	<i>Centaurea</i>	5	5	2
<i>Asteraceae</i>	<i>Cichorium</i>	2	7	1
<i>Asteraceae</i>	<i>Cynara</i>	1	3	2
<i>Asteraceae</i>	<i>Echinacea</i>	1	1	0
<i>Asteraceae</i>	<i>Erigeron</i>	1	1	0
<i>Asteraceae</i>	<i>Gaillardia</i>	1	1	0
<i>Asteraceae</i>	<i>Gazania</i>	1	6	0
<i>Asteraceae</i>	<i>Helianthus</i>	4	431	47
<i>Asteraceae</i>	<i>Helichrysum</i>	3	14	1
<i>Asteraceae</i>	<i>Heliopsis</i>	3	4	0
<i>Asteraceae</i>	<i>Lactuca</i>	2	627	67
<i>Asteraceae</i>	<i>Lonas</i>	1	1	0
<i>Asteraceae</i>	<i>Madia</i>	2	4	0
<i>Asteraceae</i>	<i>Matricaria</i>	1	1	1
<i>Asteraceae</i>	<i>Rudbeckia</i>	3	7	1

<i>Asteraceae</i>	<i>Serratula</i>	1	1	0
<i>Asteraceae</i>	<i>Silybum</i>	1	1	1
<i>Asteraceae</i>	<i>Zinnia</i>	2	25	1
<i>Balsaminaceae</i>	<i>Impatiens</i>	4	6	0
<i>Begoniaceae</i>	<i>Begonia</i>	1	1	0
<i>Boraginaceae</i>	<i>Phacelia</i>	2	2	0
<i>Brassicaceae</i>	<i>Brassica</i>	15	746	20
<i>Brassicaceae</i>	<i>Camelina</i>	3	58	0
<i>Brassicaceae</i>	<i>Crambe</i>	1	20	0
<i>Brassicaceae</i>	<i>Eruca</i>	2	4	1
<i>Brassicaceae</i>	<i>Isatis</i>	1	1	1
<i>Brassicaceae</i>	<i>Lepidium</i>	1	9	2
<i>Brassicaceae</i>	<i>Sinapis</i>	2	87	4
<i>Brassicaceae</i>	<i>Raphanus</i>	1	2	1
<i>Caprifoliaceae</i>	<i>Valeriana</i>	1	1	1
<i>Caryophyllaceae</i>	<i>Gypsophila</i>	1	1	1
<i>Caryophyllaceae</i>	<i>Silene</i>	1	1	1
<i>Crassulaceae</i>	<i>Rhodiola</i>	1	1	1
<i>Droseraceae</i>	<i>Drosera</i>	1	1	1
<i>Euphorbiaceae</i>	<i>Ricinus</i>	1	20	0
<i>Fabaceae</i>	<i>Astracantha</i>	1	1	1
<i>Fabaceae</i>	<i>Glycine</i>	1	702	11
<i>Fabaceae</i>	<i>Lathyrus</i>	14	335	12
<i>Fabaceae</i>	<i>Lotus</i>	1	18	0
<i>Fabaceae</i>	<i>Melilotus</i>	1	1	1
<i>Fabaceae</i>	<i>Trifolium</i>	2	150	40
<i>Fabaceae</i>	<i>Vicia</i>	4	692	254
<i>Gentianaceae</i>	<i>Gentiana</i>	1	6	6
<i>Geraniaceae</i>	<i>Erodium</i>	1	2	1
<i>Hypericaceae</i>	<i>Hypericum</i>	1	2	1
<i>Lamiaceae</i>	<i>Agastache</i>	1	1	0
<i>Lamiaceae</i>	<i>Betonica</i>	1	1	1
<i>Lamiaceae</i>	<i>Hyssopus</i>	1	2	2
<i>Lamiaceae</i>	<i>Ocimum</i>	1	1	1
<i>Lamiaceae</i>	<i>Salvia</i>	2	4	3
<i>Lamiaceae</i>	<i>Satureja</i>	1	6	6
<i>Lamiaceae</i>	<i>Sideritis</i>	1	2	2
<i>Lamiaceae</i>	<i>Linum</i>	2	1240	14
<i>Onagraceae</i>	<i>Oenothera</i>	1	1	0
<i>Papaveraceae</i>	<i>Papaver</i>	4	124	9
<i>Pedaliaceae</i>	<i>Sesamum</i>	1	304	225
<i>Plantaginaceae</i>	<i>Digitalis</i>	1	1	1
<i>Plumbaginaceae</i>	<i>Goniolimon</i>	4	4	4
<i>Plumbaginaceae</i>	<i>Limonium</i>	6	6	6
<i>Polygonaceae</i>	<i>Rheum</i>	2	2	1
<i>Ranunculaceae</i>	<i>Aquilegia</i>	1	1	1
<i>Ranunculaceae</i>	<i>Nigella</i>	2	2	1
<i>Rosaceae</i>	<i>Alchemilla</i>	2	2	2
<i>Rutaceae</i>	<i>Ruta</i>	1	2	1
<i>Scrophulariaceae</i>	<i>Verbascum</i>	5	6	4
<i>Solanaceae</i>	<i>Atropa</i>	1	1	1
<i>Solanaceae</i>	<i>Nicotiana</i>	1	311	191
<i>Solanaceae</i>	<i>Valeriana</i>	1	1	1
<b>Total - 28</b>	<b>80</b>	<b>173</b>	<b>6398</b>	<b>1059</b>

Table 2. National GenBank Medicinal Plants Act of Bulgaria

Family	Plant species	Number of accessions from Bulgaria
Amaryllidaceae	<i>Allium schoenoprasum</i> L.	5
Apiaceae	<i>Anethum graveolens</i> L.	3
Apiaceae	<i>Carum carvi</i> L.	1
Apiaceae	<i>Coriandrum sativum</i> L.	3
Apiaceae	<i>Foeniculum vulgare</i> Mill.	6
Apiaceae	<i>Opopanax chironium</i> (L.) Koch. subsp. <i>bulgaricum</i> *	1
Asteraceae	<i>Artemisia annua</i> L.	1
Asteraceae	<i>Centaurea cyanus</i> L.	1
Asteraceae	<i>Cichorium inthybus</i> L.	1
Asteraceae	<i>Lactuca serriola</i> L.	2
Asteraceae	<i>Silybum marianum</i> (L.) Gaerth.	1
Brassicaceae	<i>Brassica juncea</i> (L.) Czern.	10
Brassicaceae	<i>Brassica nigra</i> (L.) Koch.	1
Brassicaceae	<i>Lepidium sativum</i> L.	2
Brassicaceae	<i>Raphanus raphanistrum</i> L.	1
Caprifoliaceae	<i>Valeriana officinalis</i> L.**	1
Caryophyllaceae	<i>Gypsophila paniculata</i> L.	1
Crassulaceae	<i>Rhodiola rosea</i> L.*	1
Droseraceae	<i>Drosera rotundifolia</i> L.*	1
Fabaceae	<i>Lathyrus pratensis</i> L.	1
Fabaceae	<i>Lathyrus sativus</i> L.	10
Fabaceae	<i>Melilotus alba</i> Med.	1
Fabaceae	<i>Trifolium pratense</i> L.	13
Fabaceae	<i>Trifolium repens</i> L.	34
Fabaceae	<i>Vicia cracca</i> L.	1
Fabaceae	<i>Vicia grandiflora</i> SCOP.	2
Fabaceae	<i>Vicia pisiformis</i> L.	1
Fabaceae	<i>Vicia sativa</i> L.	209
Gentianaceae	<i>Gentiana lutea</i> L.*	6
Hypericaceae	<i>Hypericum perforatum</i> L.	1
Lamiaceae	<i>Hyssopus officinalis</i> L.**	2
Lamiaceae	<i>Salvia officinalis</i> L.	1
Lamiaceae	<i>Salvia sclarea</i> L.	2
Lamiaceae	<i>Sideritis scardica</i> Grsb.**	2
Plumbaginaceae	<i>Limonium vulgare</i> Mill.*	1
Ranunculaceae	<i>Nigella damascena</i> L.	1
Rosaceae	<i>Alchemilla achtarowii</i> Pawl.*	1
Rosaceae	<i>Alchemilla mollis</i> (Buser) Rothm.*	1
Rutaceae	<i>Ruta graveolens</i> L.*	1
Scrophulariaceae	<i>Verbascum nobile</i> Vel.	1
Solanaceae	<i>Atropa bella-donna</i> L.***	1
Valerianaceae	<i>Valeriana officinalis</i> L.**	1
<b>Total</b>	<b>42</b>	<b>337</b>

\*Protected med plants

\*\*Med Plants with special regime of protection and use – collection for trade purposes is forbidden.

\*\*\*Med Plants with special regime of protection and use – collection for trade purposes can be done only according to annual quota.

In the collection of IRGR - Sadovo are kept 32 accessions of 13 families included in the Red Data Book of Bulgaria. There are 16 critically endangered specimens, 15 endangered specimens and one specimen with extinct status.

Species such as *Achillea thracica* Velen., *Silene caliacrae* D. Jord. & P. Pan., *Alchemilla achtarowii* Pawl. and *Verbascum tzar-borisii* (Davidov ex Stoj.) Stef. - Gat., which are Bulgarian endemics, and *Verbascum*

*anisophyllum* Murb., *Goniolimon dalmaticum* (C. Presl) Rechb. f. and *Sideritis scardica* Griseb. are Balkan endemics.

All accessions in the collection were collected as a result of expeditions or through exchange with other genebanks.

### Management of PGR of medical species in the National Genebank.

The management of seed collections includes storage under optimum conditions, periodic monitoring of seed for viability and quantity, and recovery when the situation warrants. The success of long-term seed storage depends on continuous monitoring of viability and regeneration or re-collection when sample viability falls below a minimum level. The storage conditions for medicinal plant seeds are at -18°C, in airtight AL/PE-film containers (Figure 1), and seed humidity of 3 to 7% (wet basis). Seed storage is monitored regularly. Under these conditions, plant germplasm can be maintained with minimal changes for decades or a hundred years or more.



Figure 1. AL/PE-film containers

It is important to anticipate the appropriate frequency of germination control tests in the genebank. Determining the maximum storage period for each seed in the specific conditions of each seed bank is important to minimize the need for reproductions. Reproductions are very labour-intensive and costly activities, which can also have a negative impact due to the likelihood of human error.

### Storage of medical species in a living collection /in vivo/

The in vivo collection maintained at the IRGR - Sadovo numbers about 444 accessions from 21 families. Most of them belong to the families *Lamiaceae* and *Asteraceae*. Some of them have the status of protected species, included in the Red Data Book of Bulgaria, or are regulated by the Medicinal Plants Act. Figure 2 (Uzundzhalieva, K. et al., 2014)



Figure 2. In vivo collection

*In vivo* conservation is particularly important because it is the last hope for rare and threatened plant species, especially those that are on the brink of extinction. The main role of in vivo conservation and the creation of such collections is more to do with research and education than conservation itself. Conducting research and educating children and young people are very important if we are going to have knowledge of endangered plant populations.



This provides a sufficient basis for their management and the certainty that generations after us will have the knowledge and motivation to conserve them.

## CONCLUSIONS

Plant species grown *in vivo* are much more readily available for research purposes and provide many more opportunities, something that is almost impossible to achieve under on-farm conditions where species are dispersed in wild and remote populations. This also provides an opportunity to raise public awareness of rare and endangered species.

With *in vivo* conservation, consistent, easily accessible and understandable databases can be compiled with information on each species - origin, ecology, etc. On this basis, adequate conservation measures can be taken.

Clearly, the existing networks of protected areas in areas of high plant diversity will not be sufficient to ensure the long-term survival of all plant diversity. The application of an integrated approach to the conservation of PGR, combining conservation measures for plants in their natural habitats (*in situ*) with conservation *in vivo*, is the most effective tool for building a safety net against plant extinction.

Furthermore, farmers should also be encouraged to implement such measures.

Applying integrated approaches to conservation of PGR, combining on-farm and *in situ* conservation with *in vivo* conservation in living collections and gene banks is the most effective way to combat plant species extinction.

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