VISION: sustainable conservation of plant genetic resources important in the Romanian agriculture and their use in the benefits of human society and environment.

MANDATE: exploration, inventorying, collecting and studying of plant genetic resources in order to appropriately conserve them, as a precondition for food security, poverty eradication and environmental protection.

OBJECTIVES

To extend the species and genetic diversity of the three types of Genebank's collections, i.e. seeds, *in vitro*, and live plants in the field, and their conservation, according to international standards.

To promote *in situ - on farm* preservation of landraces of major agricultural species by providing people interested in growing traditional varieties, the genetic material from Genebank's collections.

To develop and adopt effective strategies to raise awareness at all levels on the importance of conservation and fast, wise and efficient utilization of plant genetic resources for food and agriculture.

BASIC ACTIVITIES

Collecting
Multiplication & Regeneration
Characterization & Evaluation
Conservation
Distribution
Documentation & Information

Collecting

General information

The activities carried out until now, on exploration and collection of plant genetic resources, brought to light a certain part of Romanian phitogenetic diversity in terms of wild and cultivated species, offering information on its large variability and location over the territory.

The collection department is focused on two distinct directions:

✓ increasing of those three types of „live" genebank's collections (seed, „in vitro" and field accessions);

✓ enrichment and diversification of „dead" collection, represented by herbarium specimens.

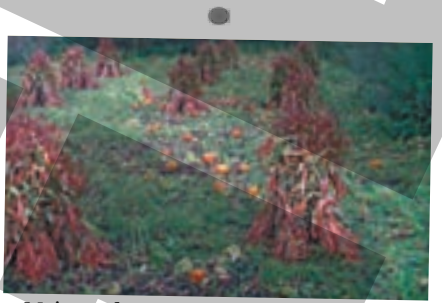
The previous phase of any collection mission consists in detailed documentation on the topographic, geological, climatic and soil conditions, species distribution, as well as on some socio-economic information, which are characteristic to the areas that is to be explored.

Collecting plant genetic resources (PGR)

Suceava Genebank keeps in collections a number of 439 plant species, classified in the following culture categories: cereals, legumes, forage grasses, forage legumes, vegetables, roots and tuber roots, industrial plants, medicinal and aromatic plants, ornamental plants. After the establishment of Suceava Genebank in 1990, we held an intense collecting activity, on a scientific basis, according to the international standards, almost yearly being organized collecting expeditions, with medium or short term period.

The collecting missions are focused on traditional varieties or local populations of cultivated plants, old varieties, removed from the culture, forms which are the most threatened by genetic erosion, and also wild forms, crop wild relatives.

Zea mays L. from Sărăsău, Maramureș



Maize and pumpkin from Cacica, Suceava

Vegetables from Dorohoi, Botoșani

Local varieties of chilies, Grădinari, Olt

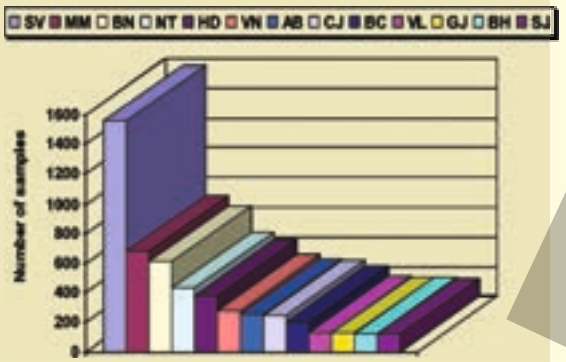
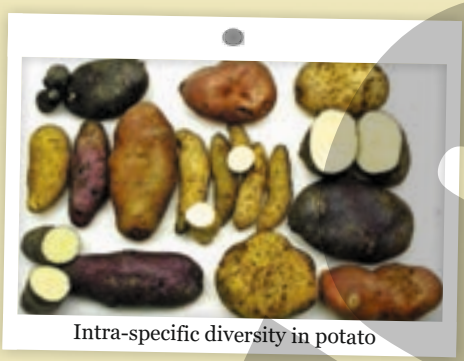


Fig. 1. Number of collected samples from the main counties of Romania


Intra-specific diversity in potato


Intra-specific diversity in beans

Table 1. The main species and number of collected samples in the period 1990 - 2014

Species	Nr. of samples	Species	Nr. of samples
Allium cepa L.	66	*Linum usitatissimum* L.	14
Allium sativum L.	38	*Lycopersicon esculentum* Mill.	58
Avena sativa L.	185	*Papaver somniferum* L.	50
Beta vulgaris L.	52	*Petroselinum crispum* Mill.	29
Brassica oleracea L.	15	*Phaseolus coccineus* L.	107
Cannabis sativa L.	28	*Phaseolus vulgaris* L.	2453
Capsicum annuum L.	127	*Pisum sativum* L.	117
Cucumis sativus L.	75	*Satureja hortensis* L.	25
Cucurbita pepo L.	238	*Secale cereale* L.	91
Daucus carota L.	26	*Solanum tuberosum* L.	322
Helianthus annuus L.	35	*Triticum aestivum* L.	174
Hordeum vulgare L.	57	*Vicia faba* L.	254
Lactuca sativa L.	41	*Zea mays* L.	2336

The collection priorities have been changed over time, so if initially we collected, only local populations of grains, legumes and potatoes, in the last years, a great variety of vegetable species, and some groups of medicinal plants, herbs, fodder and ornamentals, have been the object of our collecting expeditions, in order to improve inter and intraspecific diversity in the main crops with importance for national agriculture. In recent years, was initiated the acquisition of genetic material from local markets.

The entire area of our country was systematically explored (1015 villages from 38 counties) and a big number of seed samples and vegetative organs was gathered and introduced into seed, *in vitro* and field collections of the genebank. The accessions number collected from each county is different, the largest diversity being found in sub-mountainous and mountainous zones (Fig. 1).

As a result of more than 20 years of activity, the Suceava Genebank holds a collection of almost 16.000 accessions (crop varieties and spontaneous flora). From these, a number of 7800 samples, representing 249 species was collected by our institution, the rest being received from other sources, such as breeding institutes, botanical gardens, and universities from Romania or abroad.

Collecting missions were focused on representing, as much as possible, the genetic variability existent at national level.

Collecting cultivated plant genetic resources

In order to minimize the effect of genetic erosion process, caused by the cooperativization of agriculture and by the social-economic changes of the latest years, collecting activity was oriented on traditional varieties and old landraces which belong to the species presented in Table 1.

Taking into consideration the proportion of the crop plants collected during 20 years of activity, it could be noticed that species *Phaseolus vulgaris* L. and *Zea mays* L. are very well represented in our collections, the others being present in lower proportions, between 2% and 4% (fig. 2).

Besides the collected seeds, the information gathered from farmers who maintain these forms of plants are very important for the management of traditional cultivars. Collecting/acquisition of genetic material is always accompanied by dialogues with local people, owners of agricultural land, which preserve traditional cultivars, in order to get as much information as possible related to collected samples (the landrace spreading and traditional methods used for cultivation, drying, storing, selecting of seed material, utilization) and pedo - climatic conditions of ecological areas, also socio - economic conditions existing at households in isolated communities, these information being included in "on farm" descriptors.

Year by year, we enlarged the activity, and so far we succeeded to explore ecological zones more geographical isolated, with a rich biodiversity and a high concentration of local varieties from 38 counties and to collect a highly diversified research material from cultivated and wild flora.

Also, by exploring the same zones at various time periods, it has been observed that in Romania, after 40 years of cooperativized agriculture, the diversity of agricultural ecosystems obviously decreased. Only in certain isolated villages from intra-mountainous depressions, local varieties of the main crops are still maintained. Traditional agriculture systems, based on old landraces of wheat, maize, bean, potato, faba bean, could be found, yet, in three geographical areas: Bucovina, Maramures and Apuseni Mountains (Fig. 3)

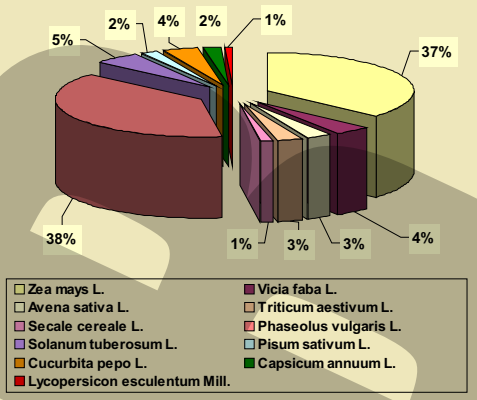


Fig. 2. The percentage of main crops collected by Suceava Genebank

Interview with local people in Măguri, Cluj

Collecting of *Avena fatua,* Rîșca, Cluj

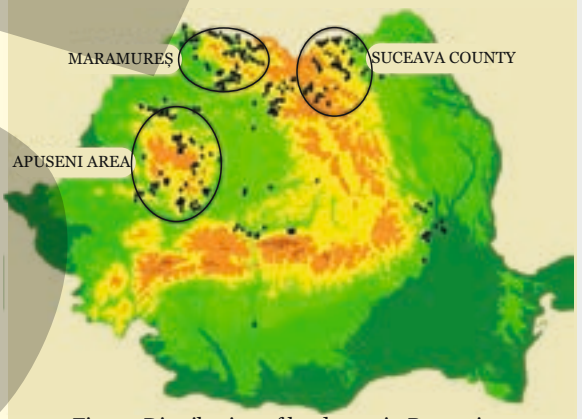
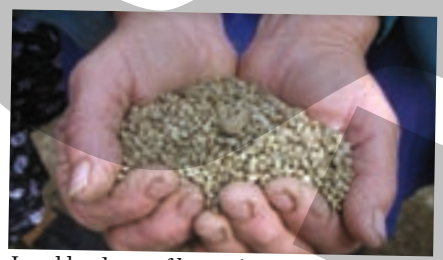


Fig. 3 - Distribution of landraces in Romania
(Map generated with Diva-Gis)

Local landrace of maize (*Zea mays* L.), Campulung, Suceava

Local variety of faba bean (*Vicia faba* L.) in Frumosu, Suceava

The harvesting of einkorn (*Triticum monococcum* L.) in Almaşu de Mijloc, Alba



Local landrace of hemp (*Cannabis sativa* L.), Cupşeni, Maramureş

Local landraces of *Zea mays* L., which until 70's years were sowed in large areas, are in a critical condition, too. As a result of strong pressure of the hybrids, a limited number of traditional varieties belonging to races Hanganesc and Moldovenesc and one subrace Hanganesc/Moldovenesc were identified only in some villages from Suceava County (Frumosu, Moldovița, Pojorâta, Vama, Deia, Putna).

A similar situation has been noticed in *Vicia faba* L., which was cultivated in many rural farms from mountainous area, being used as green and dry forms. The surface cultivated with this crop was considerable in the last years, and nowadays this species could be found only in isolated plots, in a few villages from Bucovina (Pojorâta, Valea Putnei, Lisaura).

The same problem is registered at *Triticum monococcum* L. (einkorn), a relic crop, found only in Apuseni Mountains, being mostly used as fodder, or in mixture with bread wheat (*Triticum aestivum* L.) for human consumption. Well adapted to grow under extreme environmental conditions and carrying interesting characters, including rust and powdery mildew resistances, the einkorn with winter, spring or intermediate varieties was common in many villages of Alba, Cluj and Hunedoara counties, but now just one intermediate form is cultivated by two families living in Almașu de Mijloc, Alba County, on a restricted area of less than 0.5 hectars.

The worst situation has been found in fibre crops (flax and hemp), where extinction reached 100%. Part of this material was collected prior to extinction and now is conserved *ex situ* at the Suceava Genebank.

As a result of our collecting activity, firstly we succeeded collecting of significant local and traditional varieties, because some farmers still preserve genetic variability of these local populations, by permanent cultivation and their uses, also identification of geographical areas with plant genetic resources of high biological interest, and secondly we focused on mediatization of traditional cultivars importance in conservation and sustainable use of agro – ecosystems, and strengthening the role of local communities for using and promoting these traditional forms.

Collecting of crop wild relatives (CWRs)

A major part of agricultural ecosystems is represented by spontaneous vegetal species, from which, the crop wild relatives have real economic and scientific importance.

Having this in mind, the aim of collecting CWRs is to introduce in Suceava Genebank's stored material new sources of genes in order to increase inter and intra-specific diversity of collections, and to facilitate their use into the breeding system.

Genes from wild plants have provided cultivars with resistance against pests and diseases and improved tolerance to abiotic stress.

The genetic transfer of beneficial traits from wild varieties has been so widespread, that most modern cultivars of crops contain some genes that are derived from a wild relative.

In the last 12 years Genebank organized different collecting missions covering many ecological areas, placed either in or outside protected areas, where high number of ecotypes is present, and as a result a number of 551 seed accessions was added to our collection.

Taxonomic distribution of spontaneous flora samples collected in: Maramureș Downhill, Apuseni Natural Park, Bucegi Natural Park, Ponoare Botanical Reserve, Lunca Mureșului Natural Park, Domogled – Valea Cernei National Park, shows the predominance of the following genera: *Festuca, Trifolium, Poa, Phleum, Thymus* and *Rumex*. (Fig. 4)

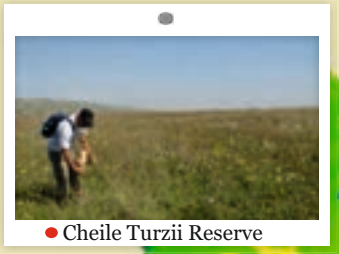
Thymus serpyllum L.

Poa media (L.) Cav.

Rumex acetosella L.

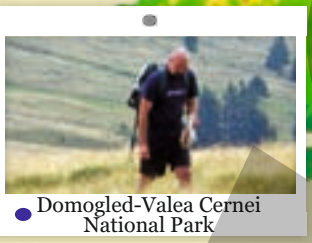
Trifolium repens L.

Fig. 4. CWR collecting sites
(Map generated with Diva-Gis)

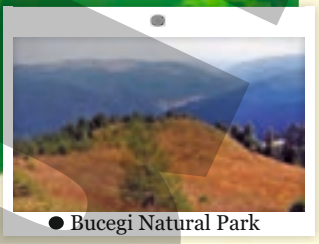


● Cheile Turzii Reserve

● Ponoare Botanical Reserve



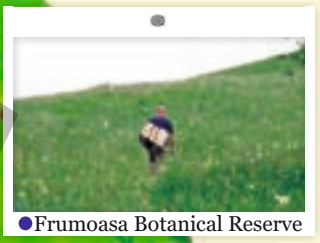
● Domogled-Valea Cernei National Park



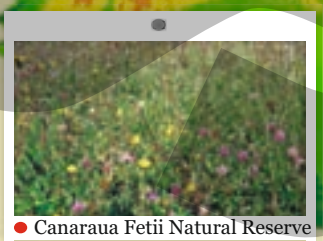
● Bucegi Natural Park

Fig. 5. Herbarium specimens collecting sites
(Map generated with Diva-Gis)

● Creasta Cocoşului Reserve



● Frumoasa Botanical Reserve



● Canaraua Fetii Natural Reserve

Colecting herbarium specimens

The Herbarium, organized according to the phylogenetic system, is constituted from whole or portions of pressed dried plants (leaf, stalk, flower etc), housed in a special room, with 24 cabinets, each one having 42 drawers, under controlled environment.

Total number of entries in the Herbarium is 1350, which covers 650 species of vascular plants, classified in 37 taxonomic orders, 120 families and 315 genera, as well as 35 species of moss and lichens representing 9 families and 20 genera.

The plant samples in the collection belong to:

- ✓ woody species (trees, shrubs, spawn - branches with leaves, flowers, rose buds);
- ✓ cultivated plants;
- ✓ crop wild relatives;
- ✓ forage, medicinal, ornamental and aromatic plants;
- ✓ ferns, mosses and lichens;
- ✓ rare or endemic plants like: *Betula nana* L., *Rhododendron kotschyi* L., *Campanula alpina Jacq.*, *Lilium martagon* L., *Leontopodium alpinum Cass*.

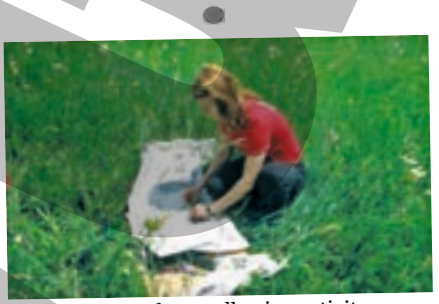
The samples of the herbarium have been collected from quite important areas, considering their geographical extent and vegetation level distribution, offering good opportunities for research (Fig.5).

The Suceava Genebank's herbarium also includes:

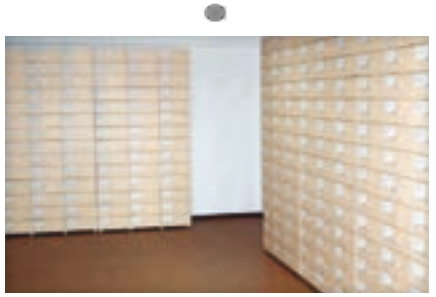
- ✓ "the auxiliary collection" with samples originated in habitats from other countries, species belonging to genres: *Ononis*, *Lathyrus* and to *Ranunculaceae* family.
- ✓ "the exchange collection" contains materials used by the bank to complete with new specimens the own herbarium, through relations with other botanical institutions.


Preparing specimen for Herbarium


Final specimen form for Herbarium


Aspect from collecting activity

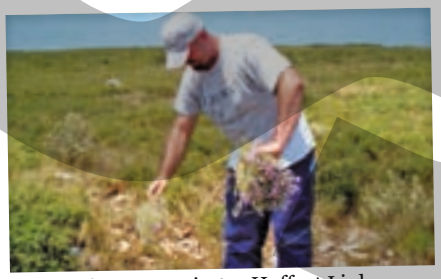

Storage drawer samples


Storage of specimens in Herbarium

Armoracia rusticana G.Gaertn., B.Mey. & Scherb.

Dictamnus albus L.

Collecting equipment



Thymus capitatus Hoff. et Link

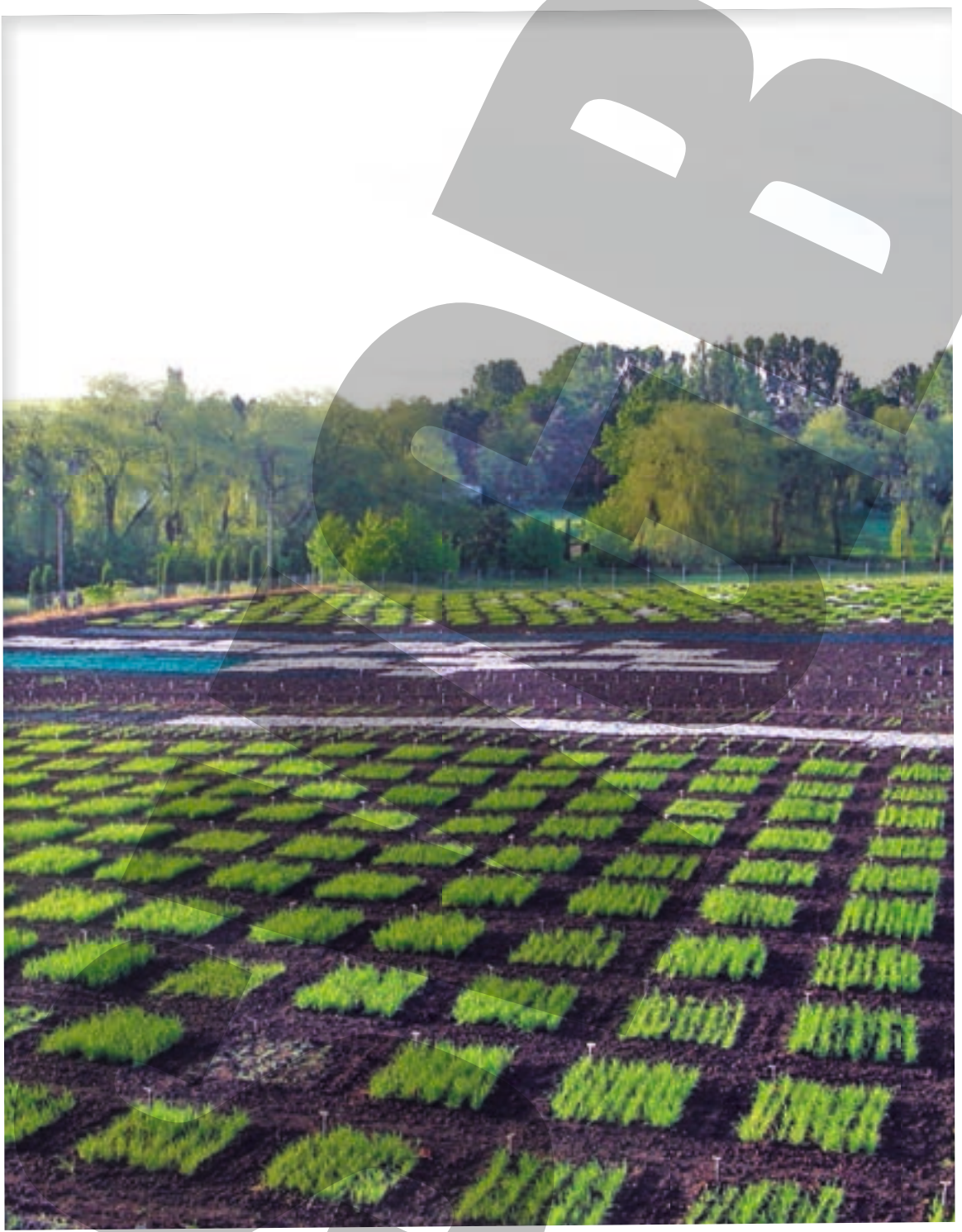
Besides wild species, cultivated plants, especially those threatened with extinction, like einkorn (*Triticum monococcum* L.), are maintained in the herbarium. These specimens are very important because they reveal morphological and ecological information of some local varieties, which are extinct, or are in danger of extinction, or have been replaced by improved cultivars.

In the Genebank database there are files dedicated to herbarium information. The access of users to the database has been simplified by means of two computerized files, which facilitate the insertion, searching and filtering of data.

The structure of the files is the following:

- ✓ the 1st file with passport descriptors, based on those recommended by IPGRI (Bioversity International), are available for all plant species:
  - institute code (INSTCODE);
  - accession number (ACCENUMB);
  - collecting number (COLLNUMB);
  - genus (GENUS), species (SPECIES), subtaxa (SUBTAXA);
  - accession name (ACCNAME);
  - country of origin (ORIGCITY);
  - location of collecting site (COLLSITE);
  - latitude (LATITUDE), longitude (LONGITUDE); elevation (ELEVATION);
  - collecting date of sample (COLLDATE);
  - biological status of accession (SAMPSTAT);
  - collecting source (COLLSRC);
  - donor institute code (DONORCODE); donor number (DONORNUMB);
  - other numbers (OTHERNUMB);
  - remarks (REMARKS);
- ✓ the 2nd file contains information about:
  - common crop name;
  - collector's name;
  - plant code.

Origanum vulgare ssp. hirtum (Link) Ietsw.



Multiplication & Regeneration

Multiplication & regeneration of plant genetic resources

An important component of the genebank's activity is represented by regeneration and multiplication of seed accessions kept into active collection, those two actions being perennial necessities of the last years.

Regeneration is the renewal of a seed sample by taking a random sample of seeds, sowing and growing the resulting plants under conditions so that the harvested seeds will show the same characteristics as the original genotype.

Regeneration of seeds is required when the germination percentage has fallen below acceptable level according to FAO/IPGRI genebank's standards.

Multiplication results in the increase of the seeds number through field propagation, getting a genetic population with the same traits like initial sample. The seed entries are going to be multiplied when seed stock is no longer available for distribution and conservation.

For most species, there are multiplication & regeneration guides which were edited by Suceava Genebank.

Seeds multiplication & regeneration is carry out in the own experimental field (1ha) and in two unheated greenhouses (0,5ha), annually it sows approximately 700-800 accessions.

For achieving of the germplasm regeneration & multiplication in the adequate conditions we have to take into considerations the following points:

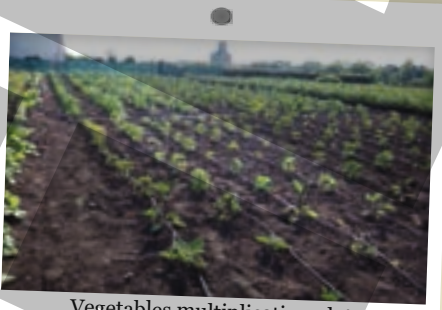
✓ Selection within the original population as a result of regeneration procedures should be minimized.

✓ The population size of the original sample should be sufficiently large to avoid genetic drift;

✓ Cross pollinating crops need additional measures to insures proper isolation;

✓ No contamination with other samples during regeneration and seed handling should be allowed.

Experimental field



Vegetables multiplication plots

Regeneration of maize accessions by controled polination

Tomato local landrace multiplied in greenhouse

Characterization & Evaluation

Recording physiological descriptors in the oat field

Harvesting of oat samples

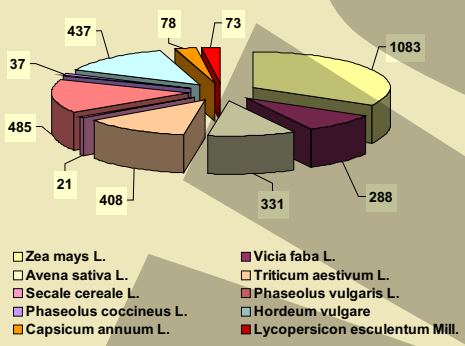


Fig. 6. Number of characterized accessions from Suceava Genebank's collections

Oat panicle attacked by *Fusarium avenaceum* (Sacc.) Sn. & H.

Characterization and primary evaluation of PGR

This activity helps to identify and describe every accession, undertaking in regeneration plots (self pollinating species) or in separately plots (cross pollinating species).

For each species it is used a minimum number of descriptors, created and developed by IPGRI (currently named Bioversity International), and sometimes complemented with traits of interest for Romanian breeders.

These descriptors contain information referring to hereditary traits that are independent or less influenced by environmental factors, the result being a quick and easy differentiation between phenotypes.

This activity is essential because provides information about the characteristics of the accessions from collection, ensuring an efficient utilization of conserved germplasm.

Till the year 2014, Suceava Genebank team, carried out the characterization and primary evaluation of 3241 accessions, belonging to 10 species (Fig. 6).

Secondary evaluation of PGR

It is a laborious activity requiring additional financial resources and manpower. Therefore, this activity is performed when there is additional funding from national and international projects.

Depending on ongoing projects and existing financial resources to prepare a work plan that includes the species and number of samples to be evaluated.

In the last five years, our curators were partners in several research projects that had the basic activities, assessment biotic and abiotic stress factors in different plants species, as follows:

✓ evaluation of oat genetic resources for resistance against *Fusarium* spp.; international project (*Avena* genetic resources for quality in human consumption, 2007-2011);

✓ determination of the maize seed susceptibility to the mycotoxine infection produced by the Fusarium spp. pathogen for the studied populations; national project (Increase of the Use Efficiency of a Main Local Maize Germplasm Fond from Romania, 2008-2011);

✓ explore the genetic variability for cold resistance in winter and spring oats by field testing; international project (Avena genetic resources for quality in human consumption, 2007-2011);

✓ resistance study of the maize plantlets to low temperatures through the determination of the cold test index; national project (Increase of the Use Efficiency of a Main Local Maize Germplasm Fond from Romania, 2008-2011);

✓ identification of some vegetable local populations (tomatoes, peppers and eggplants) tolerant to water stress; national project (Identification of tolerant genotypes to heat and water stress from traditional vegetables species suitable to specific technological system of biologic and conservative agriculture, 2010-2014).

The phytosanitary control of PGR

This stage implies two main activities:

■ Plant health monitoring during the vegetation period is accomplished in multiplication/ regeneration plots, from experimental field and in two unheated greenhouses, in order to identify the harmful organisms (fungi, insects), to establish the attack degree of diseases and pests and application of chemical measures for prevention and fighting. Plant health monitoring includes the following steps:

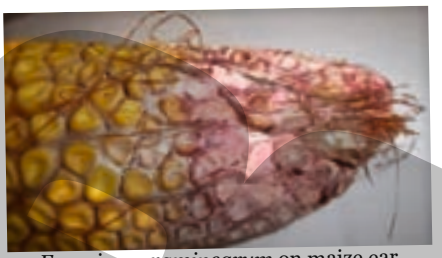
✓ timing of control period for estimation of the infection and insect damage produced to plants;

✓ sampling for analyses;

✓ identify specific micomycetes and pests that causes different symptoms on plants;

✓ estimation of attack degree of the fungi and insects on plants;

✓ application of adequate chemical measures.



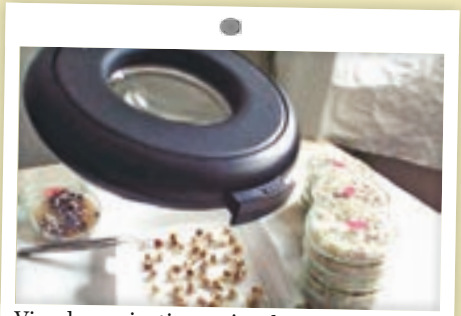
Fusarium graminearum on maize ear (Schawab) Sn. & H.

Pepper local variety growed in irigated conditions

Eggplant local variety growed in irigated conditions

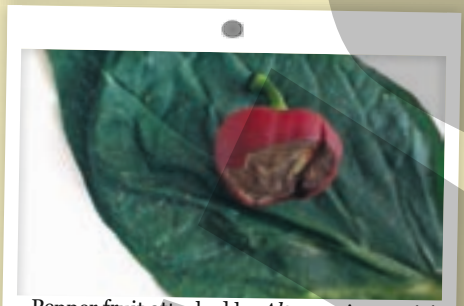
Chemical treatments application

Plants health monitoring in the field

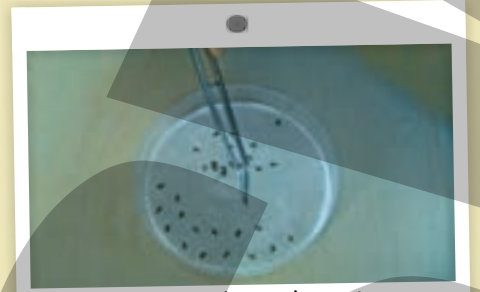


Visual examination on incubated maize seeds

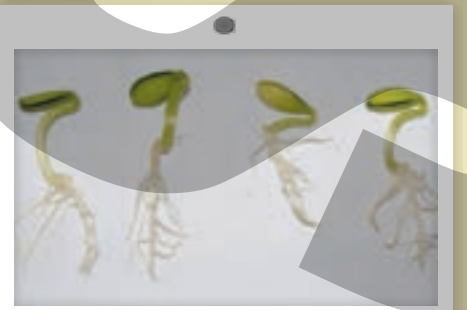
Lupin seeds attacked by *Colletotrichum gleosporoides (Penz.) Penz. & Sacc.*



Pepper fruit attacked by *Alternaria capsici* Savul & Sandu



Seed germination testing on top of absorbent paper in Petri dishes



Normal germs of *Cucurbita pepo* L.

■ Seed health testing. Seed health tests determine the status of a seed sample with regard to diseases affecting that crop or wild species. Crops are frequently infected with a range of common seed-borne pathogens that may not be visible or easily recognized during seed collection. Seed-borne inoculums reduce storage longevity and cause poor germination or spread of diseases and pests in experimental field, reducing the value of crops. Two methods are used to detect pests and pathogens: visual examination and blotter test.

✓ visual examination - The simplest method to detect diseases and pests is to examine dry seeds with the naked eye or under a low-powered microscope;

✓ Blotter test - is similar to germination tests in that seeds are placed on moistened layers of absorbent paper and incubated under conditions that promote fungal growth.

The tested seed samples are assessed as follow:

✓ health seed sample (free of diseases and pests);

✓ seed sample damaged by fungus and pests (if the percentage of seeds infected is greater than 5%, the seed lot can be considered unsuitable for conservation).

Testing and monitoring of seed viability

Maintaining adequate viability is critical to the sustainability of ex situ conserved seed collections.

In Suceava Genebank seed viability assessment is done using standard germination test, according to the International Association of Seed Testing (ISTA).

Seed samples are tested both at the entrance to the Genebank and periodically, throughout the storage period, in order to catch the moment when viability decreased below the limit accepted by standards, and as a result those accessions to be included in a rejuvenation plan. Seeds monitoring take place once at 10 years for base collection, while for accessions kept in medium term conditions (active collection) the germination capacity is controlled once at each 5 years.

Conservation

Conservation of plant genetic resources

The conservation, as central activity of Suceava Genebank, consists of maintaining the individual entries into controlled conditions in order to preserve their viability and genetic stability as long as possible. To meet these goals the Genebank is using three specific conservation methods: seed, *in vitro* and field collection.

Seed conservation

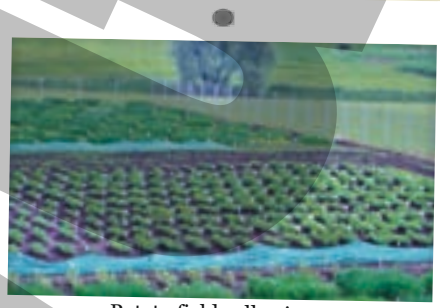
The major methodology used by Suceava Genebank to safeguard its genetic patrimony is that of seed conservation. All activities performed by the conservation section's stuff are detailed further on:

- Extending the number and the genetic inter- & intra-specific diversity of those three types of collections through biologic resources coming from:
  - ✓ collecting missions;
  - ✓ taking over breeding collections;
  - ✓ exchanges with different stakeholders from Romania or foreign countries.
- Assurance the security of collections during both, processing phases and storage, through monitoring the physical parameters in the conservation chambers by using a computer assisted program;
- Facilitating access to all *bona fide* users at genetic material through:
  - ✓ seed sample distribution to internal users for characterization & evaluation, regeneration or multiplication, viability testing;
  - ✓ seed sample distribution to external users for breeding or research purposes;
  - ✓ reintroduction of traditional varieties to farms.

The seed accessions when enter into the Genebank's collection should follow a certain sequence of operations in order to get a representative and high quality seed lot with maximum potential of viability. The figure 7 illustrates the processing steps carried out until the seed sample gets into the cold store.

Accessions from the active collection

Accession from the base collection



Potato field collection

Potato *in vitro* collection

Microtubers from potato *in vitro* collection

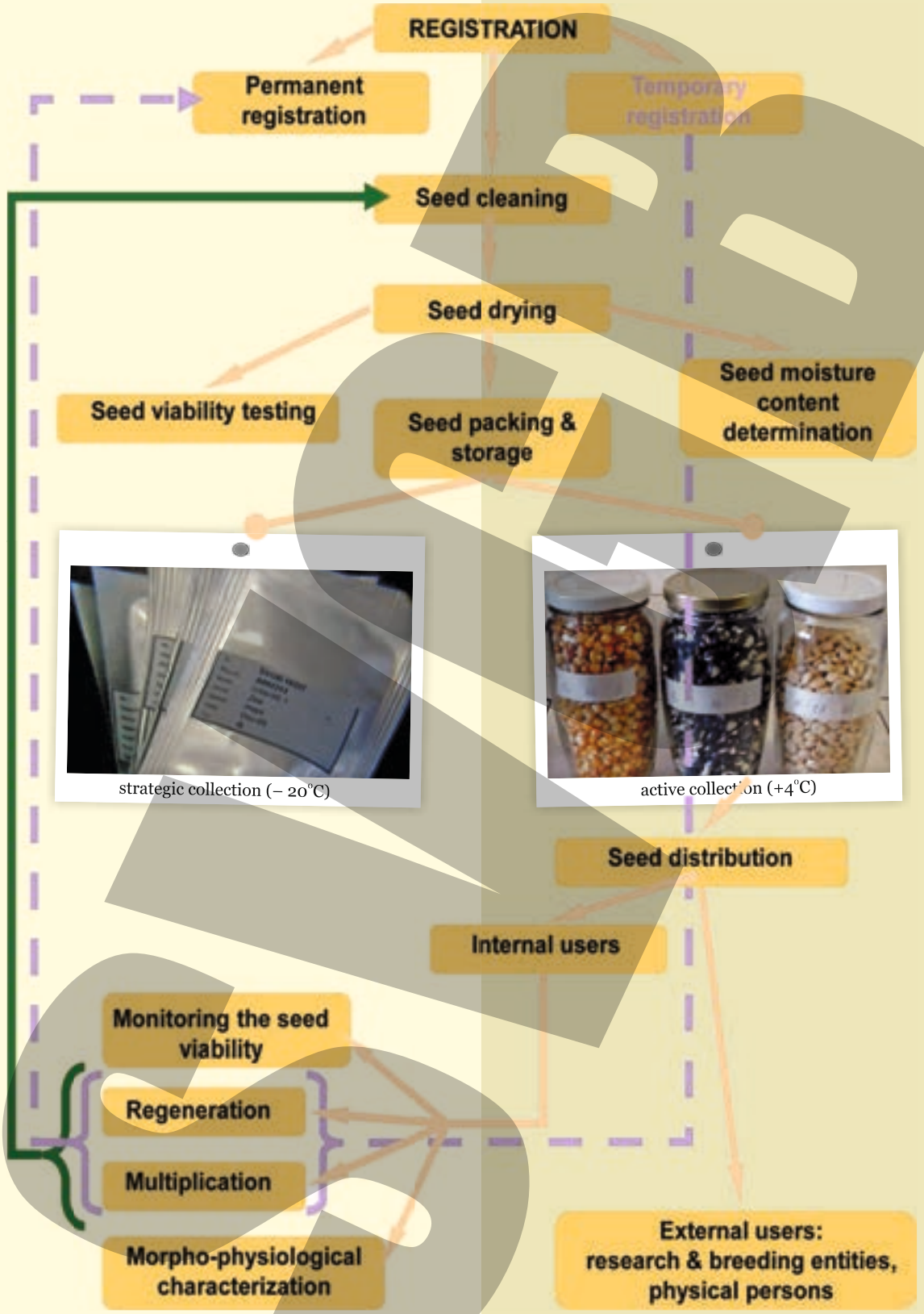


Fig. 7. The sequence of seed operations, at Suceava Genebank

The most important work having deep implications on longevity and genetic integrity of the stored material included in collections, and that's why, considered to be a storage treatment, is the seed drying operation. In this respect, the Genebank has two drying rooms where Munters dehumidifiers are running till the seed moisture content has reached between 3 – 6%, by adopting the thin layer drying procedure.

Genebank collections

At present, Suceava Genebank holds two types of collections, such as:

1. base collection (for long-term storage) that is seen like a national genetic reservoir, and its constitution has been started in the year 2001, by duplicating freshly regenerated material from the active collection, as well as from original samples brought from collecting trips. The collection consists of 4 614 accessions belonging to 44 plant species, the best represented being *Zea mays* L., *Triticum aestivum* L., *Phaseolus vulgaris* L., *Vicia faba* L., *Linum usitatissimum* L., *Hordeum vulgare* L., that are shown in the figure 8.

2. active collection (for medium-term storage) covers the genetic diversity of all relevant crops for Romanian agriculture, including their wild relatives, being destined to direct or indirect utilization. Now, the collection is comprised of 383 species with 15.625 entries, and of these with a high number of varieties forming the main part of collection are the species noted in the figure 9.

Munters seed dryer

Shelves in the drying room

Aluminum tray with seed samples

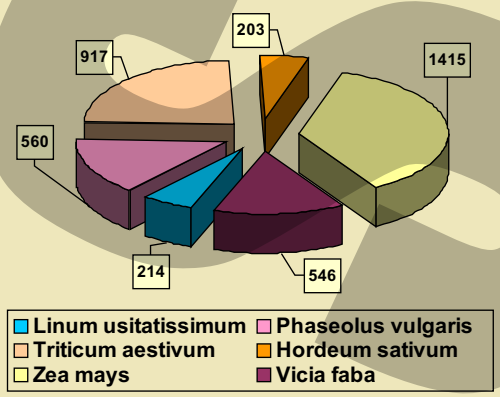


Fig. 8. Genetic material from the base collection

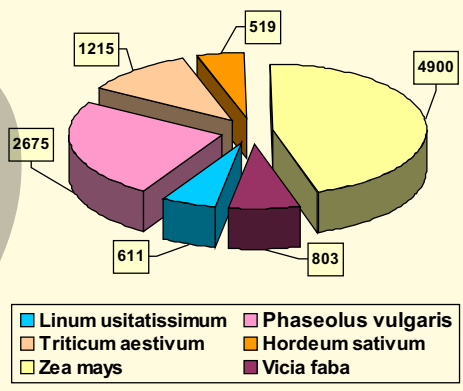


Fig. 9. Genetic material from the active collection

Table 2. The number of accessions kept in the genebank's active collection, according to their biological status and country of origin

Number of accessions		
Sample status	Origin	
	Romania	Other countries
Wild species	467	128
Local varieties	8444	641
Breeding lines	1720	846
Modern Varieties	413	850
Weeds	26	38
Unknown status	357	1695
TOTAL	**11427**	**4198**

Table 3. Storage conditions for the seed collections in the Genebank

Collection type	Conservation rooms (no. & surface)	Storing temperature	Containers used for seed packing
Base	3 cells, each of 15.9 m²	-20°C	Aluminum foil bags
Active	4 rooms, each of 23.5 m²	+4°C	Glass jars, of different capacities

Seed storage cells for base collection

Aluminium foil bags for seed conservation in the –20°C cell

Seed conservation in the +4°C room

The collection structure related to the status of sample and country of origin is reproduced in the table 2.

The storage facilities and operational conditions for seed conservation in the two collection types are presented in the table 3.

Data recording

The entire conservation department activities are reflected by the accuracy of data base filling in, what includes information on all accessions entered the two type of collections or distributed to different users. The location, date of storage, date of multiplication, number of regeneration cycles, seed moisture content, and germination capacity of each accession and each container are introduced into data file. Numerical codes are used to locate an entry within the cold store.

The information updates as well as the precise recording have implicatings on the all-subsequent activities, not only on conservation department, but also on the institution as a whole.

Field conservation of plant genetic resources

Another way for conservation of vegetative propagated species or for those with “recalcitrant seeds”, used by Suceava Genebank especially to preserve old potato varieties, is their cultivation, year by year, in the experimental field.

Potato (*Solanum tuberosum* L) is, after rice, wheat, and maize, the fourth most important crop in the world. Strong demand continues to stimulate increase in potato area and also press for the germplasm conservation of old and valuable materials, for the future use in the breeding programs.

Every family in the Bucovina area, in the 90's, at the beginning of Genebank collecting activity, used to grow 5-6 local potato varieties, each of them having special characteristics and uses. The last expeditions revealed the effects of genetic erosion, and the stringency of potato conservation, due to virus degeneration, the loosing of crop vitality and variability.

To get potato variants the collecting missions have explored 17 counties and 220 localities in Romania.

Morphological aspect varies, many of these potato populations having oval or oblong tubers with different skin and flash colors (dark violet, red, black, yellow), and high culinary qualities.

Traditionally, potato varieties have been and are still maintained in our Genebank field. The major advantage of growing the varieties in the field is the potential to verify directly the genetic integrity and morpho-physiological characteristics of the genotype.

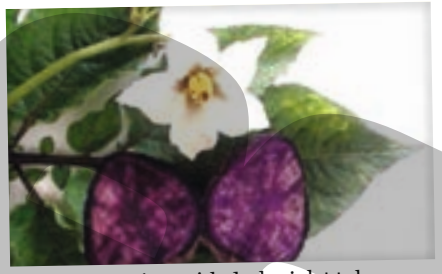
In the experimental field, during vegetation period of potato, the data on growth and development of plant are evaluated, and differences in plant size, growth habit, shoot and flower colors are recorded. The aspects regarding the resistance to environmental factors, to biotic and abiotic stress are very important, too.

Some tuber characteristics like: tubers number/plant, tubers shape, and tubers size, skin color, flesh color, number of eyes/tuber, are evaluated at the harvest time and in the storage period.

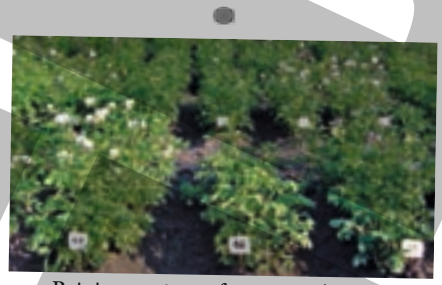
The maintenance of these potato genotypes, as field collection, and their assessment regarding the resistance to pests, diseases and environmental conditions are of great importance for the plant genetic resources conservation programme.

In vitro conservation of plant genetic resources

Tissue culture techniques are of great interest for the collecting, multiplication and storage of plant germplasm, giving the possibility to propagate plant material with high multiplication rates, in an aseptic environment. The development of *in vitro* propagation and conservation methods was imposed by the necessity to maintain plant biodiversity for so-called "recalcitrant" species and, also for those with vegetative reproduction system.

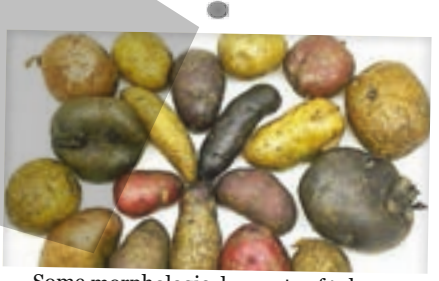


Potato variety with dark-violet tubers



Potato genotypes from experimental field collection

Potato variety with blue flower



Some morphological aspects of tubers from Suceava Genebank potato collection

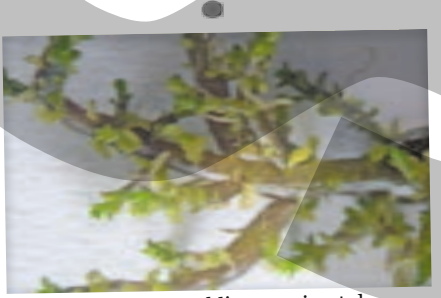
Aspects from the growing room of potato collection

Plantlets (after 39 months of conservation on the same medium)

microtubers of local potato varieties regenerated in vitro

Table 4.Composition of *in vitro* culture media for potato (*Solanum tuberosum* L.) conservation

Compounds / 1 l medium	Amount (mg)	Compounds / 1 l medium	Amount (mg)			
Medium	A, B, C, D	Medium	A	B	C	D
NH_4NO_3	825	Myo-Inositol	100			
$K NO_3$	950	Thiamine HCl	0.2			
$CaCl_2 6H_2O$	330	Pyridoxine HCl	0.2			
$MgSo_4 7 H_2O$	185	Nicotinic acid	0.2			
$K H_2PO_4$	85	Glycine	0.2			
KI	0.42	NAA	0,01			
H_3BO_3	3.1	Kinetin	0,01			
$MnSo_4 H_2O$	11.1	Benzyl adenine	0,01			
$ZnSo_4 7 H_2O$	4.3					
$Na_2MoO_4 2H_2O$	0.13	Mannitol (g)	50	40	-	-
$CuSo_4 5 H_2O$	0013	Sorbitol (g)	-	-	40	-
$CoCl_2 6H_2O$	0013	Daminozide (mg)	-	-	-	30
$FeSo_4 7 H_2O$	27.80	Sucrose (g)	20			
$Na_2EDTA 2H_2O$	37.30	Agar-Agar (g)	7,5			



Structure resembling to microtubers

In vitro medium-term conservation techniques have been developed for a wide range of plant species, but they are, still, used routinely for the genetic resources conservation of a limited number of species, such as *Musa* L. (banana), *Manihot esculenta* Crantz (manihoc) or *Solanum tuberosum* L. (potato).

In vitro conservation is the most useful way to manage cloned material collections, being the only method that allows the virus eradication, helping the production and the distribution of pathogen free genotypes, at any time.

The field collection is the main biologic material source for *in vitro* conservation methodology of potato. This technique is the second option for the medium-term storage of potato varieties, either as plantlets, or as microtubers.

Slow or low growth

Usually, the entire collection has to be subcultured on a fresh micropropagation medium in 2 – 2.5 months. The aim, for *in vitro* conservation, is to reduce growth, thus increasing intervals between two subcultures. In most cases, environmental conditions and/or culture medium have to be modified to induce growth reduction. This type of storage lowers the risk to lose material due to environmental stress and, once viruses or other infections have been eliminated, cultures can be kept pathogen free.

Various, so called, slow growth potato culture media were tested, as the same time with temperature decrease in the conservation room. Some of these media are presented in the table 4.

Observations on different characteristics (shoot length and vigor, leaves color and size, the degree of rooting and percentage of survived explants) showed a quite large variation between the potato varieties, during the period of conservation through slow growth. The buds, present in the axils of the leaves, can develop into some hypertrophied shoots, with very small leaves, or can evolve to structures resembling to microtubers.

Reduction of temperature from 20°C, in the growing room to 7-10°C, in the conservation room is commonly associated with the use of different growth inhibitors or osmotic stress in the medium, to prolong intervals between two transfers, to prevent senescence phenomena, mainly after one year of development on the same culture medium.

The results from the past showed that potato populations are different in their ability to overcome very hard growing conditions. The viability of the cultures has to be periodically evaluated. The most important characteristics in the evolution of potato stored by slow growth are: fungus and/or bacterium contamination, leaf senescence, number of viable nodes, stem length and roots development.

The plantlets used to get mini-cuttings to be subcultured on slow growth media are from the micropropagation phase. After the conservation period the biologic material is propagated on normal media where the growth is reestablished.

The capacity of shoot's apex to produce new plantlets in subculture (photos 31 - 33) is the main criterion for results evaluation, after 36 – 40 months of conservation by slow growth.

New media for slow growth are researched at Suceava Genebank, to improve the *in vitro* conservation of potato germplasm collection, especially for the less vigorous varieties.

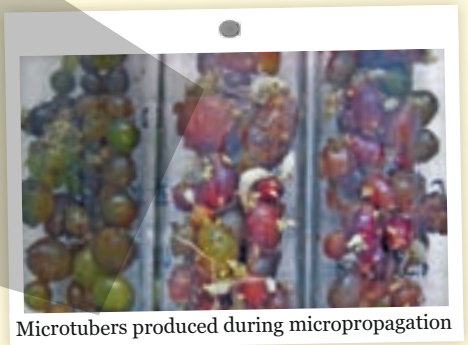
For this moment the conservation conditions of plantlets (36 - 40 months), could be synthesized as below:

- ✓ ½ MS medium;
- ✓ low content of growth regulators;
- ✓ 4 % manitol or sorbitol; or 30 mg/l daminozide;
- ✓ 2% sucrose;
- ✓ 10 h light / 24 hours
- ✓ 1000 lx light intensity;
- ✓ 7 to 10°C;
- ✓ 20 plantlets per culture medium;
- ✓ 60 plantlets per genotype

Microtubers, regenerated during the micropropagation process are prelevated in sterile conditions, and stored at low positive temperatures, closed in small glass jars.

Shoot apex, after 36 months of *in vitro* conservation through *slow growth*

Plantlets regenerated from the shoot apex



Microtubers produced during micropropagation

Details aspects of microtubers

Distribution

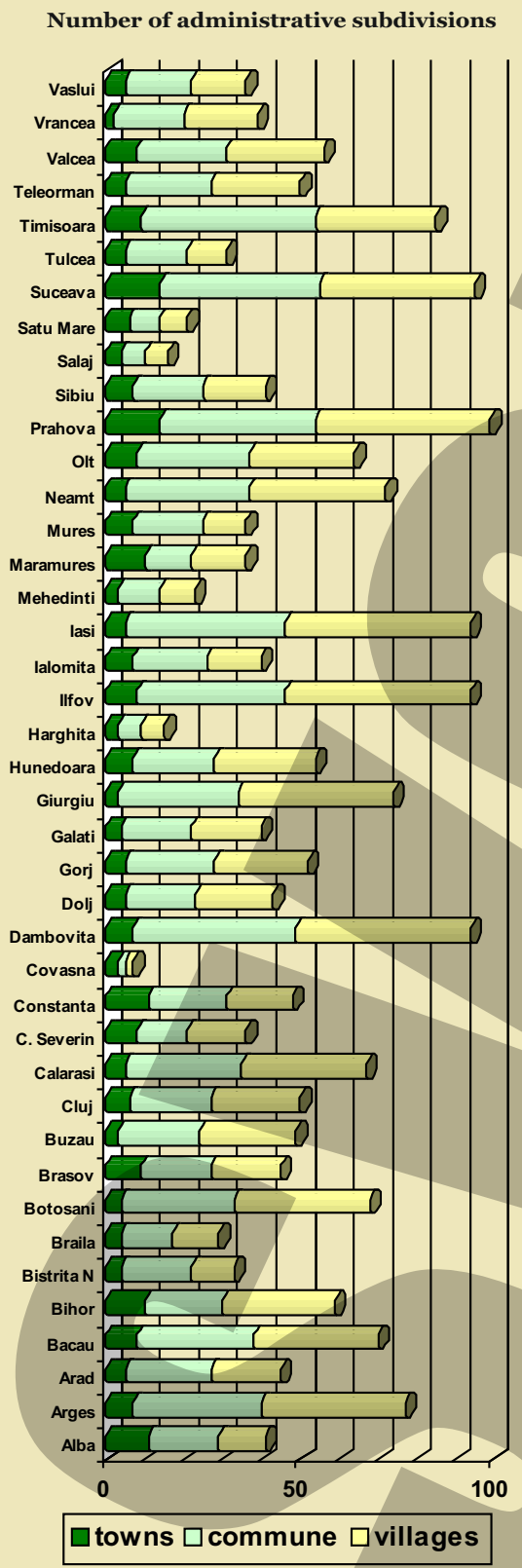


Fig. 10. Number of administrative subdivisions (towns, communes and villages) in all 41 counties of Romania, who have benefitted from the distribution of biological material from Suceava Genebank

Seed distribution

The Genebank guaranties the access to its collections and provides genetic material to all *bona fide* users, based on SMTA, when breeders and researchers are the recipients, or SVGB's Agreement in case of local growers, interested to have traditional varieties in their gardens or fields.

Distribution of local genotypes for individual persons started, in an organized way, in 2009, based on a real people's interest in having and using testier and healthier products in their daily meals.

On our website, www.svgenebank.ro, it was posted the SVGB's Agreement, and was created a link to an "order form", which is active in two periods of the year, i.e. from 15th August to 15th September for autumn cultures, and from 15th November to 15th January, for spring varieties.

All the information related to users could be found in the Genebank's database. The seed samples were sent in over 2200 localities, distributed in all 41 Romanian Counties, as is presented in figures 10.

The number of seed samples sent around Romania, since 2009 till 2014 and the most required species are presented in figures 10 and 12.

Much information related to Genebank's germplasm adaptation in different areas, and climate condition, was received from the users. Meantime, many seed samples, or vegetative organs, were received from the people wishful to contribute to this action, and to share their biological material with other persons.

The distribution of traditional varieties was and it is, also, a good opportunity in order to promote *on farm* conservation, to indentify farmers willing to grow and maintain landraces on a voluntary basis, and to enhance the use of genetic resources from Genebank's collections.

Fig. 11. Number of seed samples distributed since 2009 until 2014, in all counties of Romania

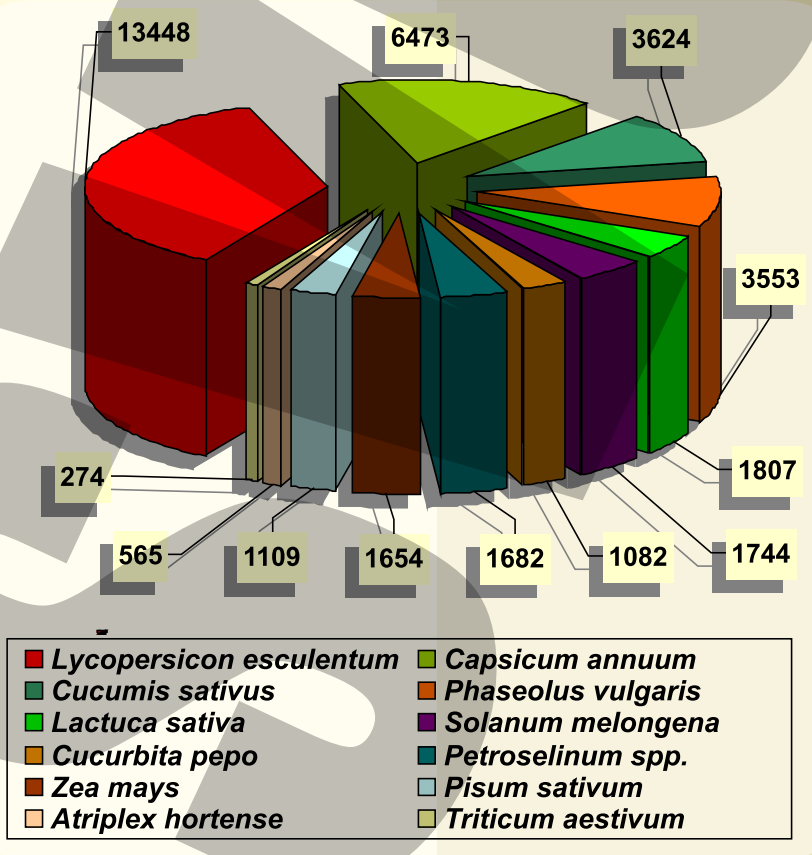


Fig. 12. Main species distributed from the active collection

Documentation & Information

General activities

For managing the huge amount of data associated with the samples included in the Genebank's, collections a suitable informatic system has been developed, year by year, here referring to specialized staff, hardware and software.

The main activities of the documentation and information office are:

- ✓creating programmes for managing all the databases;
- ✓maintaining and updating informations on SVGB site;
- ✓updating National Plant Inventory on EURISCO server;
- ✓representing Romania in the IT working group of European Cooperative Programme on PGR;
- ✓providing expert advice in the use of database of the institution;
- ✓providing technical support in IT equipments troubleshooting.

Hardware

Our local network has 12 computer stations and one server, all conected to internet.

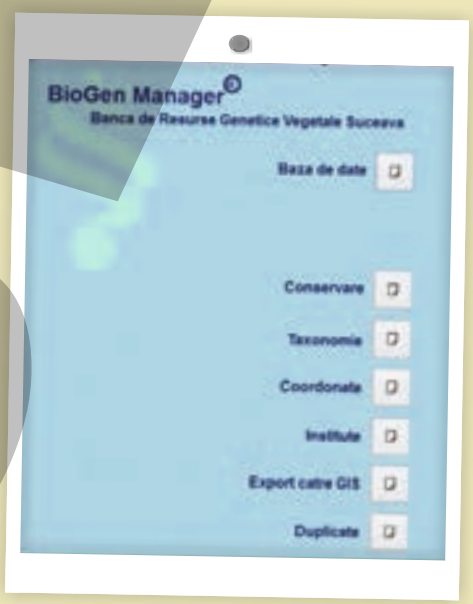
Software

Regarding the database, it has been decided to use Visual FoxPro software; this way being enabled to compute data by a modern manner using the object oriented programming technology.

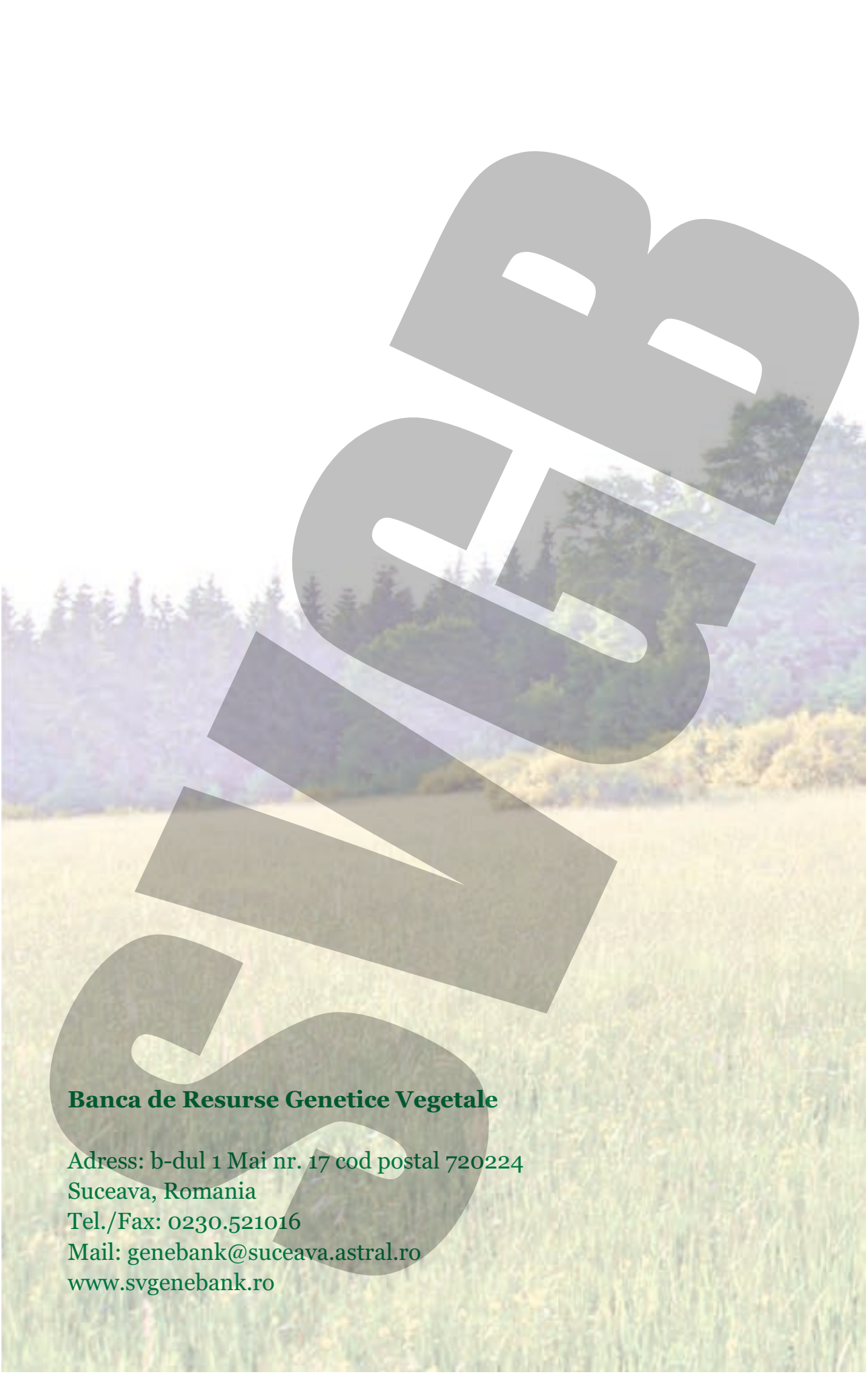
The used technologies for generating the web page are: HTML (Hyper Text Markup Language), VBA (Visual Basic for Applications), ASP (Active Server Pages), JavaScript.

Database activities:

- ✓ensuring informations integrity in the database;
- ✓improving database threads of collecting (passport and on farm), evaluation and conservation activities;
- ✓updating the national inventory passport data;
- ✓using the informatic system in the genetic material distribution process from the SVGB collection.



Training & Education



Banca de Resurse Genetice Vegetale

Adress: b-dul 1 Mai nr. 17 cod postal 720224
Suceava, Romania
Tel./Fax: 0230.521016
Mail: genebank@suceava.astral.ro
www.svgenebank.ro