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Investigation of genetic resources of cultivated plants in Lithuania

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The programme "The Scientific Researches of Plant Genetic Resources" was carried out in 2004–2008. In total, 10 reseach projects were launched on plant genetic resources at 8 institutions (Lithuanian Institute of Agriculture (LIA), Lithuanian Forest Research Institute (LFRI), Lithuanian University of Agriculture (LUA), Vilnius University (VU), Lithuanian Institute of Horticulture (LIH), Institute of Botany (IB), Kaunas Botanical Garden (KBG), Vilnius Pedagogical University (VPU)). Scientific researches of plant genetic resources (PGR) are carried out in cereal and grain legume crops, forage and turf grasses, industrial crops, vegetables, orchard plants, wild and underutilized small fruit, medicinal and aromatic plants, ornamental plants, natural and induced mutants, forest trees and shrubs. Participants of the PGR network had a little more than 28500 accessions in their field collections, and about 9200 accessions are of local origin and reach 32.2% of the total accession number. In the Plant Gene Bank long-term storage, seeds of 2385 accessions are kept.

The main purpose of this programme is to collect, accumulate and investigate native pedigree PGR – formerly and recently developed varieties of different crops, indigenous populations, valuable breeding lines, to fully investigate them, and on describing their agronomic and biological properties and characters, lodging and disease resistance, to pass them on to the Plant Gene Bank for storage and to use in practical breeding.

Key words: plant genetic resources (PGR), PGR investigation in Lithuania

INTRODUCTION

The collection of plant genetic resources of agricultural plants in Lithuania was started when the Dotnuva plant breeding station was founded in 1922 [1]. Nowadays, eight institutions are involved in the activities of collection, investigation and conservation of plan genetic resources. The National Plant Genetic Resources Coordinating Centre was founded at the Institute of Agriculture in 1993. The Baltic–Nordic PGR project was initiated by the Nordic Gene Bank (NGB) in 1994. The main objective of participation in the project was to develop the national PGR conservation network in Lithuania. This project activated the cooperation and coordination work among the education and research institutions involved in PGR investigation and conservation. In 1994–1997 the first research programme "Genetic Resources of Cultivated Plants" was pursued, funded by the State Science and Studies foundation. The genetic resources collected in Lithuania were described in the "Catalogue of Lithuanian Plant Genetic Resources" [2]. Another research programme, "Investigation and conservation of genetic resources of cultivated plants and home animals", was approved and implemented in 1998–2002. The activities of 10 institutions (universities, research institutes, botanical gardens and breeding centres) were concentrated on plant, livestock, poultry and honey-bee genetic resources [3, 4].

The programme "The Scientific Researches of Plant Genetic Resources" was continued in 2004–2008. This programme is also funded by the Ministry of Education and Science. In total, ten research projects were launched on plant genetic resources at eight institutions (LIA, LFRI, LUA, VU, LIH, IB, KBG, VPU). The main purpose of this programme is collection, investigation and conservation of native pedigree PGR.

In 2001, the law on national plant genetic resources was enacted by the Seimas (Parliament) of the Republic of Lithuania. Government of Lithuania adopted a resolution about the foundation of the plant gene bank from 1st January 2004. The functions of long-term seed storage and PGR investigation and coordination were taken over by the Plant Gene Bank which started its activity on 22 September 2004. The main goals of the Plant Gene Bank are to secure the long-term preservation and sustainable use of plant genetic resources, the accessibility, safety and particularity of information about plant genetic resources, to take part in drafting and the policy and strategy of plant genetic resources in the country and implementation of the law and other juridical acts on the preservation of the national plant genetic resources.

RESULTS AND DISCUSSION

Eight research and education institutions in Lithuania are involved in the activities of the PGR network. Duties of coordinating

centres are delegated to five institutions responsible for coordinating activities in collection, research and conservation of plant genetic resources according to different plant groups (agricultural crops, horticultural plants, forest trees, medicinal and aromatic plants, and ornamental plants). Participants of the PGR network had a little more than 28500 accessions in their field collections, and about 9200 accessions (32.2% of THE total accession number) are of local origin.

In long-term storage at a temperature of –18 °C are kept seeds of 2385 accessions (Fig. 1). The long-term seed storage is annually supplemented with new accessions. Accessions representing 87 genera and 157 plant species have been put to long-term preservation. Seeds of landraces and old varieties of agricultural and horticultural crops, advanced varieties and valuable breeding material, as well distinguished populations of wild relatives of cultivated plants and forest trees have already been stored in the long-term seed storage at the Plant Gene Bank. In 2004–2008, the a number of plant accession seeds obtained from different institutions for long-term seed storage was equal to 703 (Fig. 2). The number of accessions stored in long-term storage varied from 101 (in 2008) to 167 (2004).

The part of plant genetic resources preserved in collections was evaluated by scientists at research and education institutions. The most advanced cultivars, landraces and breeding lines

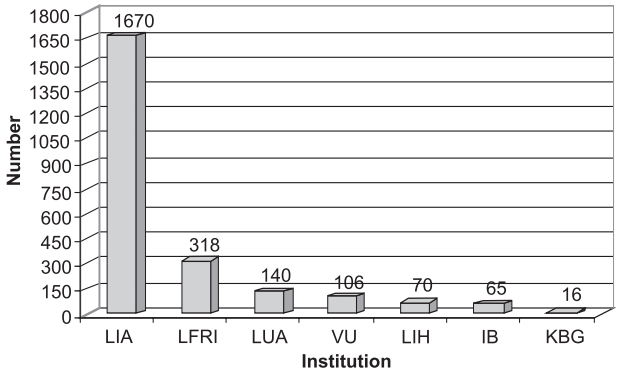

Fig. 1. Accession number provided to Plant Gene Bank long-term storage in 1997–2008. LIA – Lithuanian Institute of Agriculture, LFRI – Lithuanian Forest Research Institute, LUA – Lithuanian University of Agriculture, VU – Vilnius University, LIH – Lithuanian Institute of Horticulture, IB – Institute of Botany, KBG – Kaunas Botanical Garden

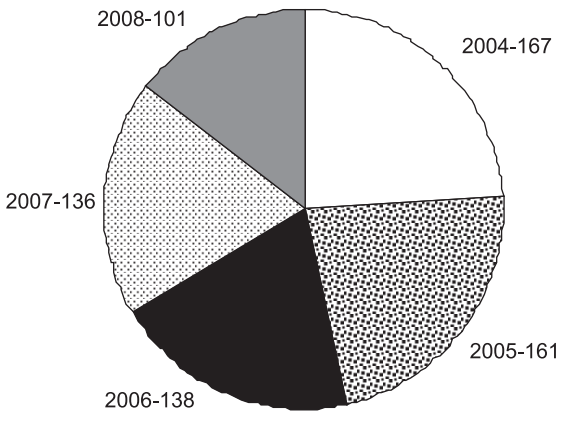

Fig. 2. Number of plant accessions stored in 2004–2008

Table. **Plant genetic resources granted the status of national genetic resources**

Plant group	Year				Total
	2003	2005	2006	2007	
Agricultural crops	–	164	222	103	489
Horticultural plants	–	65	52	–	117
Forest trees shrubs	130	–	–	–	130
Medical and aromatic plants	–	38	–	99	137
Ornamental plants	–	10	24	42	76
Total	130	277	298	244	946

were selected and proposed for granting the status of national genetic resources (Table). Such status was granted to 946 accessions. The highest number of granted accessions belonged to agricultural crops (489 accessions) and it makes a little more than 50% of the total granted accessions.

Plant genetic resources preserved at research and education institutions are used in breeding and different research and study programmes on a rather large scale. Local landraces, advanced cultivars, breeding lines, etc. are involved in plant breeding programs as donors for resistance to fungal diseases or studying plant genetics and physiology [5, 6].

Genebank collection, accumulation, investigation and conservation, both in Lithuania and abroad, are currently highly relevant and the demand for research is steadily increasing. Genebank accumulation and storage are impossible without a thorough scientific research. Many researches acknowledge that the plant genebank is one of the greatest national assets of each country. Scientific researches of Plant Genetic Resources are carried out in cereal and grain legume crops, forage and turf grasses, industrial crops, vegetables, orchard plants, wild and underutilized small fruit, medicinal and aromatic plants, ornamental plants, natural and induced mutants, forest trees and shrubs.

Cereal and grain legume crops. Genetic resources of cereal and grain legume crops are investigated and preserved at the Cereal Crop Breeding Department of the Lithuanian Institute of Agriculture and Vokė Branch of the Lithuanian Institute of Agriculture. In 2004–2008, there were investigated breeding lines, varieties, DH lines, early splitting generations and populations of winter rye, winter wheat, oats, spring barley, lupine, horse beans, peas and buckwheat. Varieties with a better adaptability were selected for further investigation, conservation and breeding programmes. The rewarding crossing with old varieties of Lithuanian origin and geographically distant varieties were made and valuable DH lines and breeding material were selected [7].

Cereal grain yield is a complex trait strongly influenced by environmental factors: severe losses can be caused by droughts, a stress common in most growing areas. The aim of the present work was to show a substantial impact of climatic conditions on the production capacity of cereal lines and varieties. The coolish and damp weather has an average influence on the develop-

ment of cereals [8]. A positive influence on the yield is exerted by a more abundant precipitation during the grain development phase, and droughts in the sprouting phase have a negative influence on grain yield. For preservation in the Plant Gene Bank and for further investigation, 205 accessions were selected.

A number soybean varieties of different origin, 58 in total, were examined in experimental fields of the Lithuanian University of Agriculture.

Forage and turf grasses. Genetic resources of forage and turf grasses are investigated and conserved at the Lithuanian Institute of Agriculture with the objective to conserve the genetic resources of forage and turf grasses by the *ex situ* method. In 2004–2007, expeditions were arranged to collect genetic resources of perennial forage and turf grasses in south and central Lithuania, Baltic littoral (Latvia) and the Carpatian regions (Ukraine), and 964 seed accessions of perennial grasses and legumes were collected. Part of wild ecotypes of perennial forage grasses and legumes collected in previous expeditions were noted for a great variation of morphological qualities and VCU valuable properties within the genus. Wild ecotypes are promising in the breeding process and valuable in terms of genetic collection accumulation [9]. Investigation of agrobiological traits of non-traditional crops in Lithuania was carried out.

There were identified by morpho-anatomical methods the following *Festuca ovina* L. s. l. species: *Festuca ovina* 22 populiations, *F. polesica* 3, *F. psammophila* 7, *F. trachyphylla* 21, *F. sabulosa* 3, *F. pseudovina* 1.

The following molecular methods were used in genetic diversity tests of clover (*Trifolium* spp.) and *Festuca pratensis Huds.* species: 1) research of genome polymorphism of meadow fescue and clover (*Trifolium* spp.) by the DNA fingerprint method; 2) search of intermicrosatellite markers for the identification of clover species and interspecific hybrids; 3) development of molecular markers for marking clover agrobiological traits; 4) research of seed protein and enzymic systems in *Festuca ovina* L. species.

A number of cenopopulations of vetches and vetchlings, 256 in total, were examined in experimental fields of the Lithuanian University of Agriculture.

Industrial crops. Investigation of genetic resources of the major industrial crops of Lithuania are carried out at the Lithuanian Institute of Agriculture and its branches, and experimental stations deal with flax and potatoes. The genetic resources of utilised industrial crops contain registered varieties, breeding material (at the final stages of breeding work), landraces and wild forms. The primary task is to collect, accumulate and investigate formerly and recently developed varieties of industrial crops, indigenous populations, valuable breeding lines, to investigate them exhaustively, and on describing their agronomic and biological properties and characters, lodging and disease resistance to pass them on to the Plant Gene Bank for storage and to use in practical breeding.

In 2004–2007, the collection test of potato genetic resources included 180 varieties, of them 12 Lithuanian varieties. The varieties were investigated and evaluated for resistance to *Phytophtora infestans*, common scab and virus diseases.

In 2004–2007, at the Upyte Research Station of LIA, some fibre flax breeding lines of Lithuanian origin and around 250 accessions from the collection nursery have been investigated for resistance to diseases (Seedling blight (*Colletotrichum lini* Boley) and Pasmo (*Septoria linicola* (Speg.) Garassini) and to negative abiotical factors. Some accessions were found to be partially tolerant to drought and could be included into fibre flax breeding programms.

Vegetables. Investigations were carried out at the Lithuanian Institute of Horticulture. The aim of this experiment was to investigate the reproduction features of selected varieties and lines for applying them to the renewal of genetic reserves as well to pick out factors influencing the biological and agricultural features and to determine the reproduction features in onions by the *in vitro* method.

The species, varieties and selected samples (tomatoes, red beets, carrots, cabbage, onions, etc.) propagated by seeds and vegetative parts were investigated. The root-crops and cabbage-heads were stored in two different places: in a vegetable compartment and a standard basement. Biochemical analyses were made after storage.

The seed plants during vegetation period were injured by a fungous disease. We studied the influence of the development of these diseases on different environmental conditions, varieties, biological individualities of the fungus and growing conditions. During vegetation, the seed plants were infested by *Alternaria*, *Cercospora*, *Stemphylium* and *Phytopthora* pathogens. Cabbages had high quality and good viability seeds.

Orchard plants. Investigations were carried out at the Lithuanian Institute of Horticulture. The species, varieties and selected samples of *Schisandra chinensis* (Turcz.), *P. lactiflora*, *P. officinalis*, strawberries, apple, sour cherry, etc. plants and vegetative parts were investigated

Schisandra chinensis (Turcz.) Baill culture *in vitro* was induced, and the impact of phytohormones BAP and IAA on the growth of microshoots in vitro was investigated. The experiment was carried out with plants of five different genotypes obtained from Lithuania and Sweden. *S. chinensis* explant reaction to cultivation in *in vitro* conditions was determined by their genotype.

The influence of nutrition media Nitsch, QL ir MS with full and half-complex mineral components on the rooting of the Lithuanian varieties 'Garbė Motinai' and 'Profesorius K. Grybauskas' of *P. lactiflora* was investigated. The nutrition medium MS with half mineral salts was optimum for *P. lactiflora* Lithuanian varieties rooting *in vitro*.

DNA polymorphism of *P. officinalis* and *P. lactiflora* species introduced into the ornamental plant collection of the Lithuanian Institute of Horticulture and the polymorphism of *P. lactiflora* cultivars and hybrids introduced or developed in Lithuania was investigated; their genetic relationship and RAPD markers were estimated.

The *Vf* gene which is responsible for scab (1–5 races) resistance in apple, by PCR was identified in the Lithuanian cultivars 'Aldas', 'Skaistis', 'Rudenis'. This gene has a stabile function in F4, F5, F6 hybrids. This gene will function in new genomes, even in scab-susceptible apples, or varieties with monogenic resistance.

Strawberry DNA fragments associated with susceptibility and resistance to red stele (*Phytophthora fragariae*) were identified and cloned using polymorphic DNA oligonucleotide primers and PCR. This primer is useful when identifying cultivars with the Rpf1 gene conferring resistance to red stele.

Different factors influenced the viability and functionality of sour cherry flowers each year. Injury of sour cherry flowers under low temperatures depends on plant genotype and flower development stage. Among the examined varieties we determined those most resistant to late frosts in spring.

Wild and underutilized small fruit. The objective of the work was to evaluate the field collection of berry plants for the contents of biologically active substances and disease susceptibility (*Rubus* subgen. *Rubus, R. idaeus* and *Fragaria*) as well as resistance to abiotic factors and the stability of properties during cultivation in the field (*Vaccinium uliginosum*). A high diversity of chemical contents was found in the native species of *Fragaria. Fragaria vesca, F. viridis* and *F. moschata* accumulate more rutin and hypericin in leaves than in fruit. Also, *Fragaria vesca* and *F. viridis* accumulate much more rutin and hyperozide in leaves than *F. moschata*. *Rubus caesius* among three blackberry species showed the highest total content of anthocyans. The most resistant to unfavourable environmental agents were the following accessions of *Vaccinium uliginosum*: *V. uliginosum* var. *viridifolium*, *V. uliginosum* var. *leucocarpum*, *V. uliginosum* morphotype of cube-shaped berries and *V. uliginosum* ssp. m*icrophyllum*. To ensure PGR conservation *in situ*, a long-term monitoring with the follow-up data analyses is necessary with regard to the amount of resources, the distribution of populations and subpopulations, resource utilisation, coexistence of other useful species as well as physical boundaries of the populations and areas to be selected for conservation.

The research object in the Kaunas Botanical Garden of VMU pomological collection was berry plants: European cranberry (*Oxycoccus palustris* Pers.), highbush blueberry (*Vaccinium* × *covilleanum* Butkus et Pliszka), lowbushberry (*V. angustifolium* Aiton) and cranberry bush *Viburnum* L. Over 360 Lithuanian and introduced accessions were investigated. Attention was focused on the properties of plants that could contribute to the success of a new crop. The general investigation schema for the accessions held in the collections consisted of phenological observations, investigations of spring frost resistance, winter hardiness, disease susceptibility, and productivity.

The best cultivars and clones of European cranberry bush were selected depending on their productivity, fruit quality, length of cluster stalk. The best 54 accessions of European cranberry that distinguish themselves by very large berries, high productivity and biochemical composition were proposed to include into the National List of Plant Resources.

Investigations under the Genetic Resources Programme have been carried out on 41 species, cultivars and clones of blue-berried honeysuckle (*Lonicera* L.) and 19 species and 30 cultivars of mountain ash (*Sorbus* L.). The study of their genetic variation and relationships among some blue-berried honeysuckle lines and cultivars was based on RAPD analysis.

Medicinal and aromatic plants. The objective of the project was to evaluate and select highly valuable and genetically stable accessions of medicinal and aromatic plants for further selection, and to revise and conserve the diversity of endangered species in their native habitats and field collections.

The endangered species of *Allium vineale* and *A. angulosum* were studied in the field collection. Investigations of plants transplanted into the field collection estimated that the ratio of flowers and bulbils in the inflorescence used for segregation of *A. vineale* varieties are not inheritable and the discrimination of varieties is purposeless. The reproductive effort of *A. ursinum* during anthesis is similar to that of monocarpic plants. A descriptor list of the morphological and chemical characters of *Thymus* × *oblongifolius* was compiled.

Essential oils of *Achillea cartilaginea* were analysed by GC and GC/MS. Two different chemical profiles of essential oils were distinguished: 1.8-cineole and camphor. The analysis of essential oil composition of *A. cartinaginea* has revealed that the content of biologically active compounds in it is high and sufficient to regard the plant as a good raw material. The major phenolic constituents identified in the ethanolic extracts of yarrow herb were chlorogenic acid, rutin and apigenin-7-*O*-glucoside. The antimicrobial activity of oregano essential oils was evaluated against fungi, yeasts and germ species. The investigations indicated different intraspecific activity of oregano accessions against the test microorganisms.

Investigations of 30 *in situ* cenopopulations of caraway showed the environmental influence on its morphological and biochemical parameters. More favourable conditions for essential oil synthesis were in pastures which are more open, than in meadows. The average essential oil content in caraway fruits from meadows was lower by 10% than in pastures. The variation of the phenotypic morphological parameters of caraway shows its high liability and capacity to survive under changing environment conditions.

The inventory of medicinal, spices and hop field collections was accomplished at the Kaunas Botanical Garden. The collections hold 400 species of medicinal plants, 113 species and 60 accessions of hops.

Ornamental plants. Investigations of the bioecological, morphological and decorative features, the phytopatological and entomological evaluation of 27 ornamental plant genera and 3119 taxa were done at four Lithuanian scientific institutions (Vilnius University, Vytautas Magnus University, Kaunas Botanical Garden and Field Floriculture Research Station).

Lists of research objects were extended supplemented with taxons that reached the minimal required age. In 2007, the tendency appeared to concentrate researches in a few genera, to accumulate data of several years and then to suggest accessions to the national gene fund register. The results of the genus *Paeonia* researches were summarized and 25 hybrids originated by E. and J. Tarvidas were suggested to the register. The virological and phytopathological features of herbaceous ornamental plant species were evaluated.

The accumulation of data on candidates to the list of the national plant genefund was continued. A preliminary list was prepared and presented to the list of the national genefund.

Natural and induced mutants. The Vilnius University collection of natural and induced mutants of various plants is preserved. Natural mutants of *Rubus idaeus* differ in economical traits, and selection among them is perspective. The high polymorphism of DNA of *Rubus idaeus* and *Pinus silvestris* plants was shown. Populations of *P. silvestris* significantly differ in heterozygosity. This may have an adaptive significance. The RAPD-PCR method was succesfully used for genotyping the natural accessions of *Rubus idaeus* collection from various points of Lithuania, and higher polymorphism was revealed by morpho-

logical markers. Several accessions may serve as a resource of economical traits.

Several hybrid combinations of barley homeotic mutants *tweaky spike* and *lax-a* were revealed as giving a very high hybrid diversity [10].

Forest trees and shrubs. Methods of forest tree genetic resources conservation and management are based on currently available scientific information and practical experience related to forest tree bioecological properties, alternation of environmental conditions and generation turnover, genotypic variability and heritability of traits.

In different forest natural regions of Lithuania, the genetic value of seed orchards is not uniform, even though they were established with the same genetic material, because the reaction of genotypes to their transfer to a new environment is variable. In the clonal archive established in the Ančia forest district of the Veisiejai forest enterprise, performance of progeny from the 1a section of the Kaišiadorys seed orchard, progeny of the 5th section of the Trakai seed orchard and progeny of the Šlienava seed orchard were superior. These progeny was most suitable for use in forest regeneration in southern Lithuania and for conservation of their genetic resources.

Assessments of the genetic parameters of English oak has shown that a significant effect of population in the start of leaf spread means that the selection of populations for *ex situ* gene conservation should be carried out not only according to intrapopulation parameters, but should also be based on interpopulation differences.

The programme of gene conservation of genetic resources of wild pear foresees establishing field collections for the assessment of their genetic value and for the further selection and reproduction. After identification and assessment of clones according to their progeny, the improvement of formation of gene resources may be continued with the aid of backward selection.
Different levels of genetic variation were obtained for black alder populations. Adaptive traits, such as budburst, showed the greatest additive variance (CV_A). The new information will be used for delineating the breeding zones for black alder and to prepare its gene conservation and breeding guidelines.

There were great intrapopulation variations in mountain elm. Elm populations from northwest and northeast were relatively more tolerant to drought which may be caused by an elm pathogen. Examples of ten perspective genotypes (*in situ*) were chosen according to the established criteria of clonal selection in protected and unprotected territories of Lithuania and planted *ex situ*. Perspective genotypes of different *Salix* L. taxa were revealed.

Since 1993, a great and successful development of the Lithuanian system of plant genetic resources was achieved. Therefore, our primary task remains to collect, accumulate and investigate formerly and recently developed varieties of different crops, indigenous populations, valuable breeding lines, to fully investigate them, and on describing their agronomic and biological properties and characters, lodging and disease resistance, to pass them on to the Plant Gene Bank for storage and to use them in practical breeding.

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KULTŪRINIŲ AUGALŲ GENETINIŲ IŠTEKLIŲ TYRIMAI LIETUVOJE

Santrauka

2004–2008 metais buvo tęsiama augalų genetinių išteklių įstatymo įgyvendinimo priemonių programa „Augalų genetinių išteklių moksliniai tyrimai“. Joje aštuonios mokslo ir studijų institucijos (Lietuvos žemdirbystės institutas, Lietuvos sodininkystės ir daržininkystės institutas, Lietuvos miškų institutas, Vilniaus universitetas, Lietuvos žemės ūkio universitetas, Botanikos institutas, Vytauto Didžiojo universiteto Kauno botanikos sodas, Vilniaus pedagoginis universitetas) vykdė dešimt mokslinių tyrimų temų. Buvo tiriami varpiniai ir ankštiniai javai, pašarinė ir vejų žolė, daržo ir sodo augalai, laukiniai uoginiai ir netradiciniai sodo augalai, dekoratyviniai augalai, natūralūs ir indukuoti augalų mutantai, miško medžiai ir krūmai. Programos dalyviai savo lauko kolekcijose turi sukaupę per 28500 pavyzdžių. Apie 9200 pavyzdžių (32,2% visų pavyzdžių kiekio) yra vietinės kilmės. Ilgalaikėje Augalų genų banko saugykloje saugomos 2385 pavyzdžių sėklos.

Pagrindinis šios programos tikslas yra vietinės kilmės augalų genetinių išteklių kaupimas, tyrimas ir išsaugojimas.

Raktažodžiai: augalų genetiniai ištekliai (AGI), AGI tyrimai Lietuvoje