

# Challenges of Gene Conservation and Breeding of Broad-leaved Tree Species in Lithuania

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The conventional programmes for conservation of forest genetic resources that were based on limiting management in protected areas have shown their inefficiency under current rapid global and local environmental change. The Multiple Population Breeding System (MPBS) concept developed by G.Namkoong is at the core of new gene conservation and tree breeding strategies that have been elaborated under the EUFORGEN Program and are currently being implemented in national programs. Specially designed networks of gene conservation areas allow us to capture the existing genetic variation and adaptiveness, their dynamic management as a way to guarantee sustainability and regeneration, that will promote genetic diversity, enhance adaptation and evolution of forest tree species and thus guarantee the success of conserving forest genetic resources and efficiency in forest tree breeding.

The Lithuanian conventional gene conservation and tree breeding system that was developed during the past 40 years has been proven inefficient and unstable. A much-needed transformation of this system has already begun. A national program for development of conservation of forest genetic resources and tree breeding is under preparation. Based on the MPBS concept, plans have been made to establish a network of *in situ* and *ex-situ* gene conservation sub-populations of all broad-leaved tree species covering all forest eco-regions, and to implement dynamic silvicultural measures there specifying the levels of conservation and tree breeding intensity by groups of species with specific biological-ecological features. Some of the current genetic reserves and progeny test plantations will be included in the new network as *in situ* and *ex-situ* gene conservation sub-populations. The national forestry policy (i.e., legislation, administrative, economic, scientific) on conservation and use of forest genetic resources should be further developed in order for the forest gene conservation and tree breeding to be successful.

**Key words:** forest genetic resources, dynamic conservation, multiple-population breeding system.

## Introduction

The conventional programmes for conservation of forest genetic resources that were based on limiting management in protected areas have shown their inefficiency under current rapid global and local environmental change.

In Lithuania, forest genetic resources are protected at the Ministry level. However, there still are no specialised laws for protection and conservation of forest genetic resources. The Law of Protected Territories, the Law of Forest, and the Law of Wild Plants, etc., do not recognise the importance of gene conservation. Botanical reserves and other types of reserves are aimed at preserving endangered species and their biotopes, but are not concerned with conserving genetic diversity as such.

Between 1994 and 2000, the EUFORGEN program promoted the re-activating and initiating of new activities regarding forest gene conservation in Lithuania. One positive result of the program was that it con-

vinced and motivated national authorities to support preparation of new legislation on gene conservation, research, applied gene conservation, and tree breeding, etc. Participation in the activities of three EUFORGEN networks helped improve the understanding of new approaches in forest gene conservation and adjust to new concepts in the preparation of new national strategies, programs and recommendations.

## Activities and present status of gene conservation and tree breeding

The applied conservation of forest genetic resources is co-ordinated and financed by the Department of Forests and Protected Territories under the Ministry of Environment of the Lithuanian Republic. The Conception Program for Forest Regeneration that was approved in 1994 includes the prospective development of the basis for conservation of genetic diversity. The Convention for Protection of Bio-diversity signed in June 1992 in Rio de Janeiro was ratified in 1995. The

State Program on Lithuanian Forestry and Timber Industry Development (approved in 1996 for the period up to 2003) and the Strategy and Plan for Action for Protection of Bio-diversity in Lithuanian Republic (approved in 1997) consider some aspects of forest gene conservation. However, there are no special national programs concerned with the development of applied gene conservation and breeding.

The following national institutions are involved in conservation of forest genetic resources and tree breeding:

- the Department of Forest Genetics and Reforestation of the Lithuanian Forest Research Institute (LFRI);
- the Lithuanian Forest Tree Breeding and Seed Management Centre (LFTBSMC); and
- the Forest Seed Control Station (FSCS).

The main emphasis of forest gene conservation and tree breeding in Lithuania includes:

- developing legislation on gene conservation and documentation of regeneration material;
- developing the strategies, programs and technical guidelines necessary for dynamic gene conservation;
- transforming the conventional national systems of gene conservation and tree breeding into a dynamic multiple population joint breeding and gene conservation system;
- continuing research on forest genetic resources;
- inventorying and documenting forest genetic resources, selecting new forest genetic and seed reserves, and controlling their status, management and use;
- breeding forest trees and managing the establishment of seed orchards;
- documenting and controlling the origin, breeding value and technical quality of forest reproductive material;
- integrating the gene conservation principles into regular, sustainable forestry.

As outlined below, there has been much recent progress in forest gene conservation and tree breeding in Lithuania.

• Some concepts of forest gene conservation were integrated into the Law on Preservation of Bred Varieties and Seed Management (1996), the Law of Preservation of Animals, Plants and Mushroom Species and Communities (1997), and the Law of Natural Flora (1998).

• In 1999, the amendments to the Law of Protected Territories on forest gene conservation were proposed and currently are under adoption.

• In 1999, the amendments to the Law of Forests on forest gene conservation were proposed and currently are under adoption.

• The Law on Conservation of Plant Genetic Resources currently is under preparation.

• In 1996, the Regulations on Forest Genetic Reserves were prepared and adopted. The Regulations were updated in 1999 and were adopted in 2000 (Gabriavičius, Danusevičius 2000).

• From 1995 to 2000, inventory of the existing gene and seed reserves, plus trees, and seed orchards, was accomplished etc.

• In 1996, the set of PC databases on Lithuanian forest genetic resources was created (in 1999 these databases were joined and a common database on all forest genetic resources was developed).

• The National Register of Forest Genetic Resources is issued annually based on information in the updated databases.

• In 1997, the Catalogue of Lithuanian plant genetic resources that includes a chapter on forest genetic resources was published in the English language.

• In 1997, new Seed Regulations were prepared and adopted (Česnavičius et al 1997) These regulations deal with establishment and use of forest genetic resources, and with documentation of forest regeneration material in accordance with OECD Scheme for the Certification of Forest Reproductive Material Moving in International Trade.

• A national collaborative project titled "Plant genetic resources" (which is the State Research Program, 1998-2002) was started.

• A research project titled "Eco-genetic variation and gene conservation of broad-leaved tree species" (1999-2003) was started.

• A national forest gene conservation and breeding program based on MPBS currently is under preparation.

• Based on EUFORGEN recommendations, the national technical guidelines for dynamic *in-situ* conservation of Norway spruce and non-rare Noble Hardwoods were prepared and adopted.

• Seed zoning of Scots pine, Norway spruce and English oak was prepared and adopted.

• The National Gene Bank of Plants, that includes the forest genetic resources, was established with technical assistance and financial support from the Nordic Gene Bank.

During the past 40 years a wide network of units for conventional gene conservation and tree breeding

has been created in Lithuania. However, the main efforts were made on Scots pine and Norway spruce. At present, the network of gene conservation and tree breeding of broad-leaved tree species consists of: 1 strict genetic reserve (202 ha), 89 genetic reserves (913 ha, fig. 1), 46 seed reserves (422 ha), 415 plus trees, 1 archive of clones (4 ha), 130 progeny test plantations (203 ha) and 4 seed orchards (5 ha).

droughts, insect outbreaks, and windfalls, or due to natural succession of tree species. It seems that we have already experienced some of the negative consequences of global climatic changes. Based on experience and on new understanding of the general gene conservation objectives and principles, the shortcomings of the conventional static forest gene conservation system are outlined below:

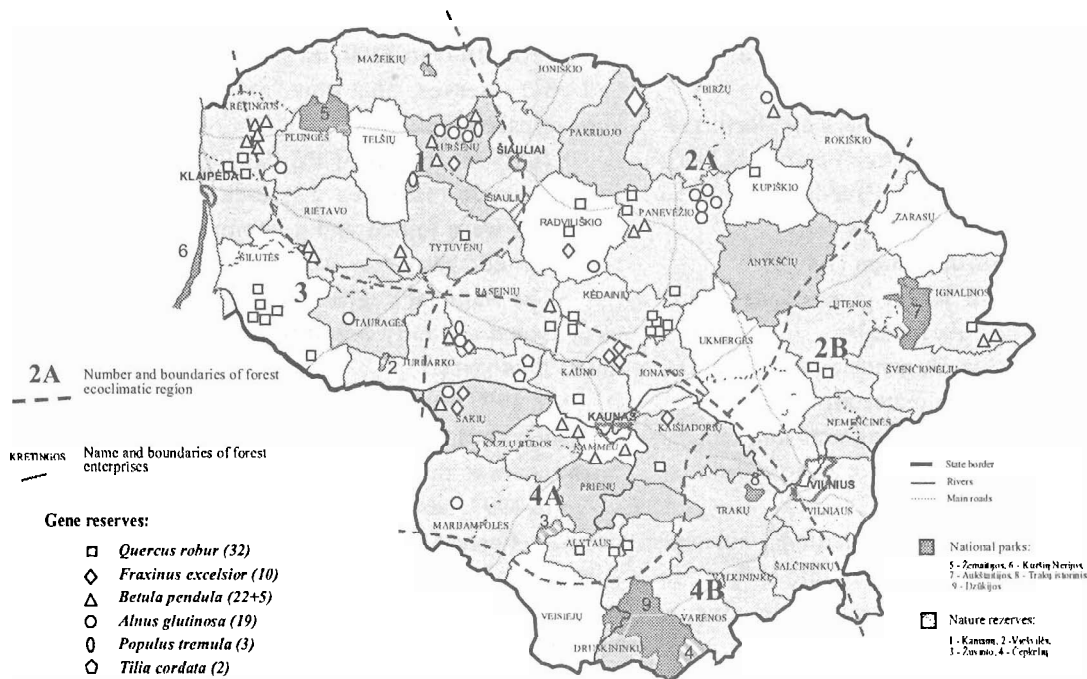


Figure 1. The network of forest gene reserves of broad-leaved tree species in Lithuania

Strict genetic reserves were designated to preserve sustainable development in communities of species over a large forest area (more than 200 ha) aiming to achieve an environment that is as natural as possible for the evolutionary forces to act upon. Genetic reserves were designated to preserve genetic diversity of a species over environmentally heterogeneous sites on comparatively small areas (less than 30 ha) by restricting silvicultural activities. Recent work in establishing conventional gene conservation network that safeguarded important populations from cutting should be considered as the first step in creating a functional gene conservation system. Now we need to proceed further in order to secure sustainable gene conservation.

Unfavourable environmental conditions in recent years showed drawbacks in the conventional *in-situ* conservation system. Over half of the coniferous genetic resources and a portion of the broad-leaved genetic resources were lost due to combined action of

1. In the past, the non-interference approach and management restrictions were based on preservationist conservation objectives were not adequate to the current rapidly changing environment. Therefore the:

- a) sustainability of gene conservation populations is low;
- b) regeneration of conservation population is not guaranteed;
- c) existing genetic constitution is 'frozen'; and
- d) use of regeneration material from the best genetic resources in forestry is very limited.

2. Natural genetic diversity was insufficiently represented from geographical, population, ecological, and generation viewpoints. In the past:

- a) the principles, criteria and methods used for selecting and sampling of genetic resources were based mostly on the commercial values of tree breeding objectives;
- b) geographical patterns of tree species variation were not considered in selecting, sampling, and conservation;

c) geographical eco-climatic patterns were neglected; and

d) only the oldest generation is represented in gene conservation areas.

3. Legislation, financing, and publicity were insufficient to accomplish gene conservation objectives. In the past, there was:

a) insufficient legislation for gene conservation of forest tree species;

b) inadequate financing to conserve a large number of gene conservation populations;

c) insufficient education of foresters on gene conservation; and

d) insufficient public awareness.

These shortcomings call for the urgent development of a new system for sustainable and secure gene conservation that would be flexible enough to cope with rapid environment changes and expansion of human activities into forestlands.

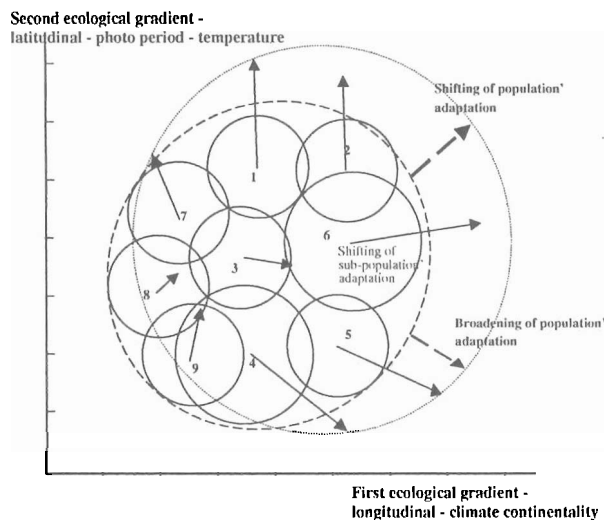
### Challenges of gene conservation and tree breeding

The prime objective of gene conservation is to ensure the continuous survival, adaptation, and evolution of a species over unlimited number of generations in a continuously changing environment. The objective of breeding of broad-leaved tree species is to improve human utility features, including: (1) quality of stem and wood, (2) growth, (3) resistance and adaptability while conserving a sufficient genetic variation for each successive cycle of long-term selection, taking into consideration future changes in breeding priorities and climate. In order to achieve these objectives, it is necessary to promote the maintenance of a broad genetic variation and create favourable conditions for adaptation of each species. The dynamic approach in gene conservation was advocated in multitude of papers (Namkoong, 1984, Hattemer and Gregorius, 1990, Danell, 1993, Eriksson et al., 1993, Varela and Eriksson, 1994, Eriksson, 1994, Hattemer, 1995, Eriksson, 1995, 1997, 1998, Koski et al., 1997, Baliuckas and Pliūra, 1998, Rusanen, 1998, Pliūra, 1998, 1999, Jensen, 1999, etc.). One general prerequisite for successful evolution is the regeneration of the gene resource conservation population. Therefore, active measures are recommended where there is a difficulty in maintaining the gene resource population over successive generations. Active silvicultural management is recommended to promote and conserve Noble hardwoods in all types of forests (Rotach 1999).

The conventional static forest gene conservation system of the past is being transformed into a dynamic one that is based on the **Multiple Population Breeding System (MPBS)** concept. The MPBS concept combines secure and sustainable conservation of forest genetic resources, preparation for possible eco-climatic changes, and efficient tree breeding (Namkoong 1984, Danell 1993, Eriksson et al 1993 ). The corresponding MPBS scheme for conservation of Scots pine in Lithuania has already been developed (Eriksson & Pliūra, 1997). Basically, the same parameters would hold for broad-leaved tree species, with some adjustments based on the results of ongoing research. Knowledge of the pattern and degree of the genetic variation of broad-leaved species that are present in a region will serve as a basis for the national forest gene conservation strategies and programs.

In accordance with the MPBS, a gene resource conservation/breeding population should consist of 10-20 small sub-populations, each with an effective population size of 50 genetic entries. At present, it is assumed that at that size of breeding-gene conservation population (750-1100 individuals) the alleles of frequencies down to 0.01 (uncommon + common alleles) will be sampled and that will provide sufficient genetic variation for both long-term sustainable gene conservation and long-term breeding purposes (Danell, 1993, Varela and Eriksson, 1995). In creating breeding lines (with the 10 best families in each) alleles of frequencies up to 0.25 are involved and are sufficient to guarantee diversity and sustainability of new stands, and to provide high genetic gain. In order to capture the adaptations that already are present in different eco-climatic conditions, selecting and sampling should sufficiently cover geographical patterns of species genetic variation as well as eco-climatic conditions. The essence of dynamic gene conservation by using the MPBS concept is to promote adaptation by exposing the gene resource population to natural selection and in turn to evolution in a variety of directions (Eriksson et al., 1993) (Fig. 2). Therefore, a gene conservation network of both natural *in situ* sub-populations as well as synthetic *ex situ* ones ought to be established over a broad array of eco-climatic conditions.

Gene conservation populations should be intensively managed to improve the adaptation of each sub-population, to increase genetic differences between them, to guarantee their sustainability during all periods of ontogenesis, to insure continuous regeneration of the population of the target species, and to protect



**Figure 2.** Changes in distribution of sub-populations over two ecological gradients: shifting of sub-populations adaptation in variety of directions, shifting and broadening of population adaptation range.

against all types of damage. In order to minimise costs gene conservation ought to be carried out jointly with tree breeding and genetic studies.

Recently EUFORGEN has recommended the levels (milestones) of gene conservation activities that ought to be followed on a step-by-step or parallel manner. These levels are:

- a) defining eco-geographic zones (“seed zones”);
- b) performing inventories of actual species distribution and conservation status;
- c) promoting afforestation using local material within an eco-geographic zone (unless genetic knowledge suggests otherwise);
- d) implementing *in situ* conservation measures in managed stands with at least 100 regularly fruit producing trees each;
- e) selecting at least 30 stands throughout the distribution area and in nature reserves (5-10 in Europe) in order to create a European network of gene conservation stands;
- f) designating one gene conservation stand in each eco-geographic zone; and
- g) establishing *ex situ* conservation and seed production areas.

In order to successfully develop these gene conservation measures and to transform the conventional national system of gene conservation and tree breeding into a dynamic multiple population joined breeding - gene conservation system, we must develop and adopt legal and policy instruments that promote and encour-

age forest gene conservation in state and private forests. We must:

- completely integrate the concepts of dynamic forest gene conservation into existing and new national legislation on forestry, the environment, nature protection, etc. (e.g., the Law on Preservation of the Natural Flora, the Law of Protected Territories; the Law on Conservation of Plant Genetic Resources, and the Law of Forests);
- develop and adopt the national strategies for dynamic gene conservation, national programmes and a logical framework for applied conservation and tree breeding for all tree species;
- develop and adopt criteria and indicators on sustainable forest management relating to forest genetic resources in order to use them in policy analysis;
- develop the recommendations (guidelines) which ensure that forest management and regeneration respect the gene conservation requirements;
- create a special national fund for supporting the activities on research and applied forest gene conservation and tree breeding;
- develop and adopt legal and economic policy instruments in support of using appropriate forest reproductive material in state and private forests;
- establish a designated sub-unit at the Department of Forest Protected Territories under the Ministry of Environment of the Lithuanian Republic in order to monitor and co-ordinate at national level the activities on forest regeneration, gene conservation and tree breeding;
- advance knowledge among foresters on the basic genetic requirements and gene conservation;
- increase the level of general public awareness on forest gene conservation;
- seek international collaboration in joint research projects with financial and technical support or assistance by other countries or international organisations and funds.

### Constructing breeding and gene conservation program

In accordance with occurrence, distribution, population size, social status, stage in ecosystem, and economic importance in Lithuania, broad-leaved tree species could be divided into 3 groups (Table 1). Some of species characteristics differ from those that species demonstrate in Nordic countries or West Europe.

Three different gene conservation and breeding programs that are based on MPBS and evolutionary ap-

**Table 1.** Characteristics of broad-leaved tree species in Lithuania

Species	Area, Thous ha	Occurrence	Distribution	Population size	Social status	Stage in ecosystem	Econ. importance
<b>1. Common social broadleaves</b>							
<i>Alnus glutinosa</i>	109	Common	Wide spread	Medium	Social	Pioneer	+
<i>Betula pendula</i>	375	Common	Wide spread	Large	Social	Pioneer	++
<i>Fraxinus excelsior</i>	51	Common	Wide spread	Medium	Social	Secondary	++
<i>Quercus robur</i>	34	Common	Wide spread	Medium	Social	Climax	++
<i>Popula tremula</i>	52	Common	Wide spread	Medium	Social	Pioneer	+
<b>2. Uncommon social broadleaves</b>							
<i>Acer platanoides</i>		Uncommon	Wide spread	Small/scat. trees	Soc./asoc.	Secondary	-
<i>Fagus sylvatica</i>		Rare	Localised in SW	Small	Social	Climax	-
<i>Quercus petraea</i>		Rare	Localised in S	Small	Social	Climax	-
<i>Tilia cordata</i>		Uncommon	Wide spread	Small/scat. trees	Social	Climax	-
<b>3. Rare asocial broadleaves</b>							
<i>Carpinus betulus</i>		Uncommon	Spread in SW-SE	Small	Asoc./soc.	Secondary	-
<i>Malus spp.</i>		Rare	Wide spread	Scattered trees	Asocial	Secondary	-
<i>Pyrus spp.</i>		Rare	in S, SW	Scattered trees	Asocial	Secondary	-
<i>Prunus avium</i>		Rare	Spread in W	Scattered trees	Asocial	Secondary	-
<i>Sorbus aucuparia</i>		Uncommon	Wide spread	Scattered trees	Asocial	Secondary	-
<i>Ulmus spp.</i>		Uncommon	Wide spread	Demes	Asoc./soc.	Secondary	-

proach are under preparation for these groups of broad-leaved tree species. These three different programs are: (1) for common social broad-leaved tree species of high economic importance that are going to be intensively bred and conserved; (2) for uncommon social broad-leaved tree species; and (3) for rarely occurred asocial broad-leaved tree species of minor economic importance that are going to be conserved with low-intensity tree breeding.

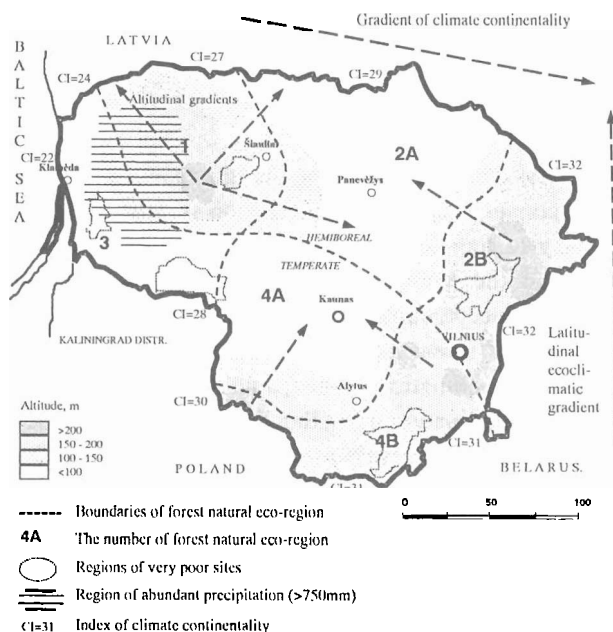
The networks of gene conservation sub-populations are under creation along defined eco-climatic gradients and forest eco-regions (=regions of provenance) in Lithuania (Fig. 3).

For the common social broad-leaved tree species that are under long-term breeding in Lithuania (*Alnus*

*glutinosa*, *Betula pendula*, *Fraxinus excelsior*, *Quercus robur*), intensively managed 10-12 *in-situ* (2 in each of 6 breeding zones) and 4-7 *ex-situ* gene conservation-breeding sub-populations are planned. For the uncommon social broad-leaved tree species (*Quercus petraea*, *Tilia cordata*, *Acer platanoides*, *Fagus sylvatica*), 6-10 *in-situ* and 2-3 *ex-situ* gene conservation sub-populations should be established over breeding zones where the species are more abundant. For the uncommon rare asocial broad-leaved tree species of minor present economic importance (*Ulmus spp.*, *Prunus avium*, *Carpinus betulus*, *Malus spp.*, *Pyrus spp.*, *Sorbus aucuparia*), 3-6 *in-situ* and 2-3 *ex-situ* gene conservation-breeding sub-populations as a progeny test plantations or clonal archives in breeding zones where that species is more common are planned. The additional planting of these rare species could be done in openings within the forest or in forest margins in *in-situ* gene conservation populations designated for gene conservation of other broad-leaved tree species.

*In-situ* gene conservation sub-populations are under establishment on areas that already have the most suitable conventional gene reserves (Fig. 2). In the regions that have no suitable gene and seed reserves some new sub-populations should be selected. The sub-populations should represent: (a) the forests of the main eco-regions (regions of provenance or breeding zones); (b) marginal populations; (c) populations valuable for breeding; (d) endangered populations; (e) populations that have rare or distinctive features; and (f) populations growing under specific ecological conditions.

The networks of *ex-situ* sub-populations are being established as regular progeny test plantations (Fig. 4). In order to safeguard the evolutionary potential of the species and increase the efficiency of tree breeding the initial population size of each *ex-situ* gene conservation-breeding sub-population should be of 100-150



**Figure 3.** Eco-climatic gradients and forest eco-regions in Lithuania



**Figure 4.** The network of progeny test plantations – *ex situ* gene conservation sub-populations of common broad-leaved tree species (*Alnus glutinosa*, *Betula pendula*, *Fraxinus excelsior*, *Quercus robur*) in Lithuania

genetic entries. The genetic diversity of *ex-situ* sub-populations could be increased using the material from neighbouring eco-regions and countries. The new generations will be created using open pollination or crossing the best 50 individuals selected within the best 50 families.

The combination of methods for mating, testing, selecting and creating production populations in constructing tree breeding-gene conservation *ex-situ* programs is selected based on species eco-genetic peculiarities, species economic importance and financial possibilities. The principal combination of methods is shown following the connecting dashed line in Figure 5. Due to financial limitations there is no realistic to plan to use a high number of families from plus trees and representatives of natural sub-populations, and some very intensive methods (e.g., artificial crossing, testing of vegetative progeny, vegetative propagation, etc.) in the first cycles of breeding broad-leaved tree species. The principle scheme (with some alternatives) of multiple-population long-term breeding-gene conser-

vation of non-rare broad-leaved tree species in Lithuania is presented in Figure 6.

### Conclusions

After clearly recognising the drawbacks of the national conventional gene conservation system, we are on the way to transforming it into a dynamic one that is based on the Multiple Population Breeding System (MPBS) concept, combining secure and sustainable conservation of forest genetic resources, preparation for possible eco-climatic changes, and efficient tree breeding. The combination of methods considered in the new national gene conservation-tree breeding-program that are under preparation is chosen based on new knowledge in population genetics, species eco-genetic peculiarities, and corresponds to the financial possibilities of the country. The networks of *in situ* and *ex-situ* gene conservation sub-populations covering all forest eco-regions are under establishment for the main broad-leaved tree species and are planned for the rest of the species.

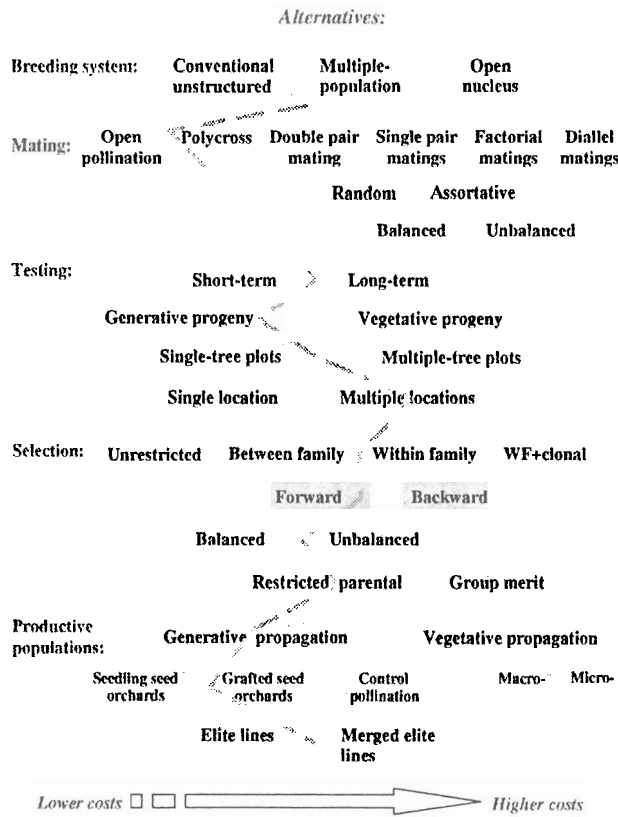


Figure 5. Scheme of selecting alternative methods in constructing of tree breeding programs for different tree species

The changes in national forestry policy on conservation and use of forest genetic resources are clearly recognisable and promote the development of forest gene conservation and tree breeding in Lithuania.

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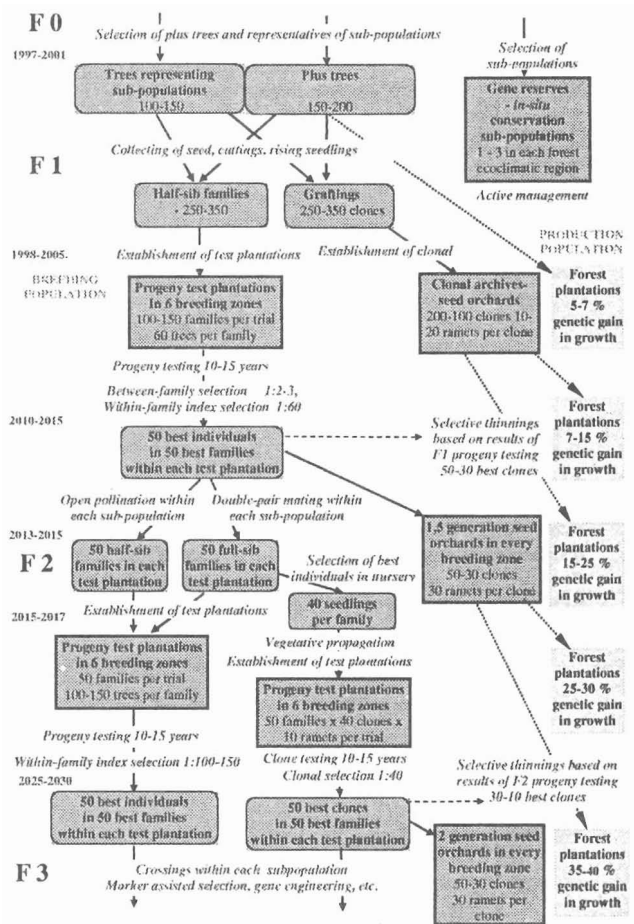


Figure 6. Scheme of multiple-population long-term breeding-gene conservation of non-rare broad-leaved tree species (*Alnus glutinosa*, *Betula pendula*, *Fraxinus excelsior*, *Quercus robur*) in Lithuania

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