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GENE POOL CONSERVATION AND TREE IMPROVEMENT IN SERBIA

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This paper presents the concepts applied in the gene pool conservation and tree improvement in Serbia. Gene pool conservation of tree species in Serbia includes a series of activities aiming at the sustainability and protection of genetic and species variability. This implies the investigation of genetic resources and their identification through the research of the genetic structure and the breeding system of individual species. Paper also includes the study of intra- and inter-population variability in experiments - provenance tests, progeny tests, half- and full-sib lines, etc. The increased use of the genetic potential in tree improvement

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in Serbia should be intensified by the following activities: improvement of production of normal forest seed, application of the concept of new selections directed primarily to the improvement of only one character, because in that case the result would be certain, establishment and management of seed orchards as specialised plantations for long-term production of genetically good-quality forest seeds, and the shortening of the improvement process by introducing new techniques and methods (molecular markers, somaclonal variation, genetic engineering, protoplast fusion, micropropagation, etc.).

Key words: conservation, gene pool, tree improvement

INTRODUCTION

There are many reasons why it is so significant to protect forest genetic resources and why this is rapidly becoming increasingly critical. Any action that reduces the forests or destroys a part of forest populations can lead to undesirable and dangerous consequences. There are many destructive factors, e.g. insects, diseases, fuelwood cutting, clear cutting for agricultural purposes, urbanisation, wildfires, storms and other natural disasters, which unfortunately often occur integrally. The practice of removing autochthonous forests and their replacement with coniferous species, most often pine and spruce, in most cases results in the reduction in broadleaf genetic base to the level of risk to the species. The most unfavourable situation happens when these phenomena occur in forest populations of trees with disjunctive populations or limited ranges. It is especially alarming for the ecotypes with limited habitats or sometimes for the entire range of endemic species. In such circumstances, it is not only gene complexes or genes that are at risk, but also the entire populations, as in extreme cases it may lead to the species elimination. As a consequence, some forest tree species or provenances are reduced to only a few hundreds of survived trees, e.g. yew (*Taxus baccata* L.), Turkish hazel (*Corylus collurna* L.), black poplar (*Populus nigra* L.), etc. The ability of tree populations to adapt to environmental changes is the basis for the stability of forest ecosystems. Forest tree species can adjust to environmental signals through only three mechanisms:

- (1) Physiological adaptation. The organisms adjust to a new environment through internal self-regulating mechanisms. If the individual tree carries many different alleles (i.e. has a high degree of heterozygosity) it can better buffer against fluctuating environmental conditions.
- (2) Epigenetic adaptation. This heritable adaptation that is not based on classical (Mendelian) rules. Epigenetic adaptation may be triggered by environmental signals, may regulate gene expression over generations and may have long-lasting effects. Mechanisms similar to genomic imprinting are presumed to exist in forest trees.
- (3) Collective adaptation. The population adjusts its genetic composition by eliminating those individuals (i.e. through natural selection) that have

exceeded their physiological adaptive range. The range of collective adaptation is determined by the extent to which the gene pool can respond to environmental factors.

In all three mechanisms, genetic diversity plays a key role: without genetic diversity, all adaptational processes are inconceivable. However, it is still debated among geneticists how much genetic diversity is needed for the long term existence of populations (BOOY, 2000).

Any form of major destructions, such as wildfires, insect calamities, diseases and storms, can imply the gene loss; however the consequences following the disaster are equally serious. If forest populations are regenerated spontaneously, there is no danger of the loss of gene resources, but if the destruction is so great that forests cannot be regenerated, a serious gene loss can occur. This type of general destruction is rather common in arid regions; therefore it is an important cause of the loss of genetic resources.

The objective of numerous programmes of forest species improvement is to increase production and to enhance the characteristics of the basic material intended for various, first of all economic, purposes in which the quantity and quality of anthropogenically established populations are of primary importance. The rate of improvement is measurable from the very beginning, as it is expressed in relation to the parameters of the base population, i.e. it is compared to all expressions of the species in nature. Simultaneously, improvement can be defined as a measure of a more effective selection, or as a possibility of removing the undesirable genes from the local population.

The improvement potential, or the selection scope, depends primarily on the species genetic variability during its evolution in nature. The greater the variety of genotype structures among individuals, the so-called wider genetic variability, the greater the chances generally speaking for the species or the population to reproduce and survive, or to adapt to variable environmental conditions. For tree breeding programmes, this simultaneously means a higher potential for the selection of the desired genetic composition.

Breeding programmes in forestry include only a part of genetic variability, selecting and therefore favouring only the gene combinations that satisfy the defined goal or goals. The end results are high-yield, but phenotypically uniform trees, which are a highly susceptible material of reduced tolerance and resistance to pests and diseases. This means that it is essential, just as in other natural and renewable resources, to develop the methods for the conservation of the genetic potential of natural populations of tree species.

Previous researches point to some effects of gene pool conservation when the tree improvement programme covers a small population, i.e. local populations within the species total range. (ISAJEV *et al.* 1990; 1997; 1999; 2003.).

Forest trees may have 20.000 up to 50.000 genes. These genes determine growth, form and physiological response of trees among many other traits. Certain genes may make trees grow faster, straighter or enable them to thrive in harsh

environments. Admittedly, this is a very simplified view and in reality genes interfere with their environment and themselves. And even non-coding DNA – rashly termed as junk by many geneticists – has recently proved its evolutionary significance such as functional pseudo genes (HIROTSUNE *et al.* 2003) or rib switchers (CECH 2004).

Genetic resources occur at different degrees of genetic variability. The examples of genetic resources, in decreasing order of genetic variability, are: natural communities, *ex situ* conservation, plantations, selected seed nurseries, and clonal seed-orchards, figure 1.

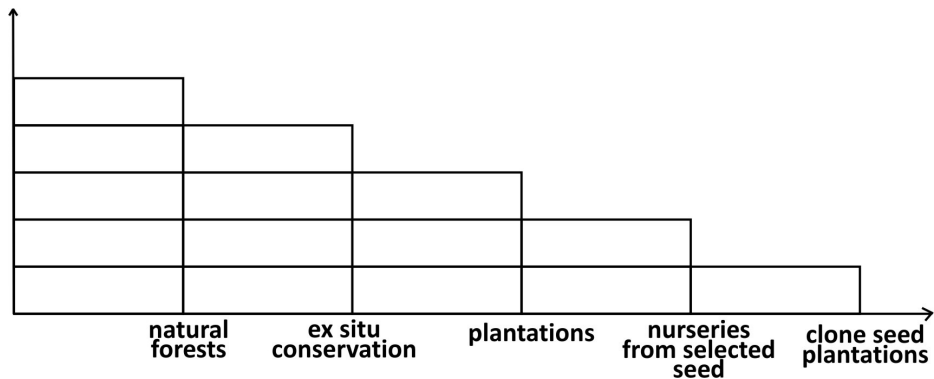


Figure 1. Scheme of the relation between the yield or improvement rates, at different levels of genetic resource variability

It is of crucial importance to assess how much of the original genetic variability should be retained or protected in long-term improvement programmes to ensure a sufficient application flexibility of the improved material. Forest tree species, in which the basic breeding methods are applied – selection, hybridisation, and more rarely induction of mutations, have different genetic compositions and different reactions to the above methods. For this reason, the genetic structure, reproductive biology of each individual species, and the potentially applicable improvement systems should be defined, but they do not exist for the majority of species at the global scale. The population genetics study can suggest the consequences of the restriction in the genetic diversity occurring when the improvement is directed to the specific purposes. The success in improvement and gene pool conservation depends on the implementation of improvement rules - sampling, i.e. forms of selection, immanent characters of the species and inbreeding effects, as well as on the interaction of environmental factors and biological traits of the species and populations, i.e. on the knowledge of the optimal and critical regions of their occurrence.

Choice of samples and selection

In the selection of samples from the selected population, the genetic structure of the selected material will be less variable than that of the base material, i.e. the macro population with natural evolution. This is because some gene combinations may be lost or deliberately excluded in the selection of desirable characters defined by the breeding programme. The selection in the succeeding generations will induce further reduction in genetic variability. For this reason, the sample size in forest tree gene pool conservation is of fundamental significance, because it decides the further work on the tree improvement programme planning and realisation. Instead of a simple criterion in the choice of sample size, which does not exist unfortunately, it is necessary to apply the experience earned in the same or similar activities in which the achievements in population or quantitative genetics are more developed.

In the selection of samples from the initial population, the frequency of genes in their alleles is reduced. The first to be lost are the genes which are rare or recently generated. The significance of the loss will depend on the sample size, i.e. on the number of individuals per sample, on the ratio of individuals – trees from which the seeds were collected, and on the fact how much the samples reflect the distribution of species in different site conditions. In small populations, the succeeding generations express the most significant reduction in genetic variability, especially when their size does not change over time. The study results of the relation between population size and genetic variability in *Drosophila*, Table 1, are indicative as they also refer to other animal and plant species. They unquestionably confirm the principle that the smaller the population, the more genetic variability is lost in the succeeding generations.

If the population size increases in the second or the succeeding generations, the increase in variability is not directly proportional to the population physical increase, but it will be slowed down.

Table 1. Genetic variance in small populations of constant sizes for the number of drosophila generations

Population size	Percentage of genetic variance remaining after 1, 5, 10, 100 generations				
	1	5	10	100	
2	75	24	6	<<1	
6	91,7	65	42	<<1	
10	95	77	60	<1	
20	97,5	88	78	8	
50	99	95	90	36	
100	99,5	97,5	95	60	

In forest trees, the generation shift lasts much longer, so the expression of the effects on genetic variability will consequently be slower. However, regardless of the difference between the trees and *drosophila*, in the case of small populations, the

basic effect of sample selection on genetic variability should be taken into account, because it is the crucial part of any breeding programme.

Population size and inbreeding

Potential inbreeding and its rate must be taken into account in the selection of population(s) or individual trees for genetic resource conservation. The rate of inbreeding can be expressed as the decrease in heterozygosity per inbreeding generation. In the case of self-fertilisation, as the strongest form of inbreeding, half of the loci will become homozygous in each generation, so the inbreeding coefficient, "F" is 0.5. The effect of different degrees of inbreeding expressed as the increase in homozygosity is presented in Diagram 1.

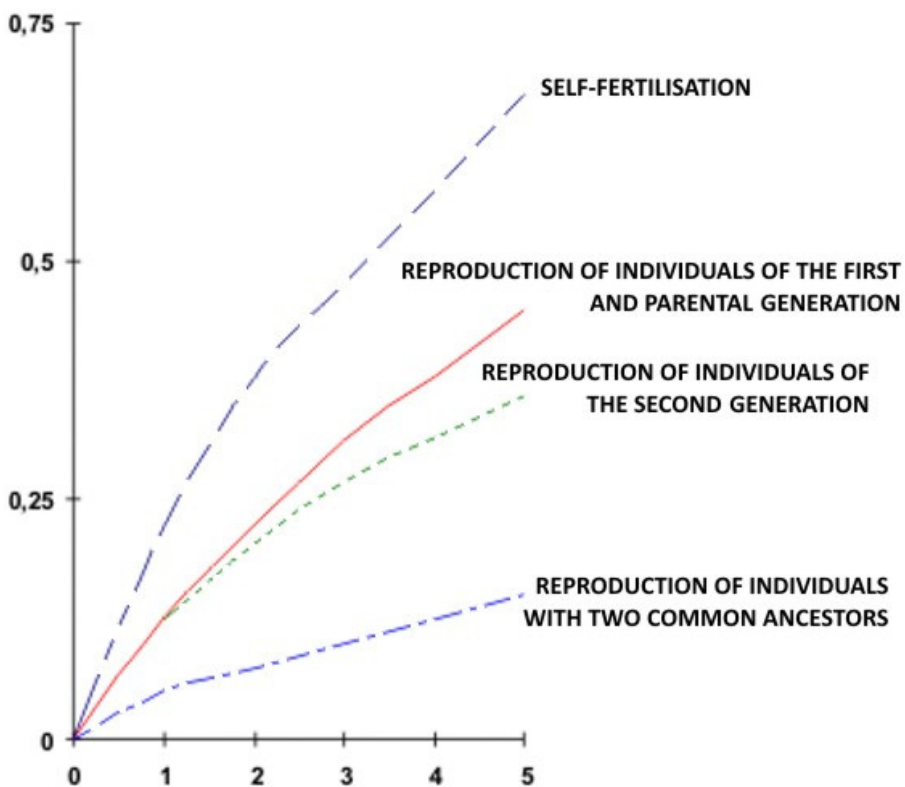


Diagram 1 Homozygosis increase in inbreeding, after Underwood

Inbreeding can have a negative effect on the characters important for cultivation and on the efforts to conserve the genetic variability. To reduce the unfavourable effects, inbreeding in the parental and established populations should be kept to a minimum, except in rare cases, when it is consciously planned. To this

aim, it is necessary to apply the rules in the selection of parent seed trees, both regarding their number and regarding the distance between them. The adequate sampling depends on the knowledge on improvement systems and the potentials for their implementation in natural conditions - individual or mass selection, selection for continuous or discontinuous characters and the scope of natural seed dispersion. The typical situation in many countries includes seed collection from natural forest populations for the establishment of nurseries or *ex situ* stands for conservation or for provenance tests. In the selection of population size, the tolerant inbreeding rate ranges from 1 - 2 %, i.e. inbreeding coefficient $F = 0.01 - 0.02$. The justification for this tolerance is based on the experience of numerous researches dealing with the lowest possible population size sufficient for the continuous survival and reproduction. The size of such a population is called effective population size (N_e). According to numerous studies, it is the population size of 50 unrelated individuals or the effective population, that will result in inbreeding percentage 1 (one), which is mathematically represented by the formula: $F = 1/2 N_e$, where F - inbreeding coefficient, and N_e - effective population size. Based on this equation, if $N_e = 50$, F is close to 0.01.

Forms of genetic resource conservation

In basic terms, there are two standpoints of genetic resource conservation of which one is taken from agriculture and implies high technology, the techniques of accelerated improvement of specific characters, gene preservation in gene-banks, and the establishment of seed orchards as the dominant form of gene conservation (ISAJEV *et al.* 1997).

Forest trees may have 20,000 up to 50,000 genes. These genes determine growth, form and physiological response of trees among many other traits. Certain genes may make trees grow faster, straighter or enable them to thrive in harsh environments. Admittedly, this is a very simplified view and in reality genes interfere with their environment and themselves. And even non-coding DNA – rashly termed as junk by many geneticists – has recently proved its evolutionary significance such as functional pseudo genes (HIROTSUNE *et al.* 2003) or rib switchers (CECH 2004).

The other standpoint starts from the fact that, by natural regeneration, the populations are supplied with the optimum and with the stability of genetic resources, and that the conservation of the natural supply is the best and the sufficient conservation method. The advantage of natural regeneration and conservation of the existing gene pool is especially supported by the Scandinavian countries. Although in popular terms, none of the two approaches provides the complete idea of the present situation, or satisfies the future needs. Both approaches start from the hypothesis that the genetic resource is static and that conservation should only postpone the gene and species loss. However, as the resources are always in a dynamic state, the efficient conservation requires an active programme of utilisation and conservation.

Genetic resources, from the historic aspect, have changed through forced methods and the optimum "adaptability" has rarely been achieved. The current state is the initial condition for the adaptation and other evolution forces, so neither the forms of conservation of natural selection results, nor the professional and scientific actions in the fields of tree selection and genetic resource conservation should be neglected. Any comparison of the experiences of different countries, which reflect the scientific attitudes on the conservation methods expressed primarily through their legislation, is difficult because of different meanings adopted for the terms "natural reserves", "national parks", "old-growth forests", "live archives", "tree improvement" or "silviculture". The precise and harmonised terms should take into account the time and space dimensions and should not neglect the forest natural dynamics. The question is whether the meaning of gene pool conservation and nature conservation is the same and also what should be done for the professional public not to get the impression that it is sufficient to perform only the adequate measures of passive protection over a great number of regions.

Forest populations can be classified into three groups which are further characterised in the process of gene pool conservation:

- natural populations which are not under normal forest management,
- managed natural populations,
- managed artificial populations.

Table 2 presents the categorisation of forest populations and their role in gene pool conservation.

Table 2. Characteristics of forest population types

Type	Physical size	Genetic variation	Operational approach	Genetic approach
Protected areas	large	high	passive	static
<i>In situ</i> gene reserves	small	high	active	dynamic
Natural stands for wood production	very large	declining	variable	indifferent
Improvement study populations	very small	high and stratified	active	dynamic
Populations for seed production	very small	moderate or low	active	selective
Artificial stands for wood production	very large	uncertain	active	ambivalent

In Serbia, the forms of gene pool conservation are classified into two basic groups, depending on the fact whether the conservation is carried in the existing, natural populations, or in the established specialised populations.

1. gene pool conservation *in situ* – selection of mature spontaneous populations and implementation of a special management regime:
 - natural reserves,
 - national parks,
 - seed stands, and
 - tree groups or individual trees;
2. gene pool conservation *ex situ* – establishment of specialised populations, such as:
 - arboretums,
 - live archives,
 - tests provenances,
 - progeny tests,
 - seed orchards.

Gene pool conservation *in situ*

In situ conservation is performed by the designation and protection of the stands of desired species or the complexes in the aim of preventing further losses, due to the reduction in their physical size and/or genotype numbers, which usually result from human activities.

The process of developing and implementing an adapted, *in situ* conservation programme may thus be divided into the following seven activities which need to be accomplished (ROTACH, 2000):

- collection of relevant information;
- selection of target species and setting of priorities;
- establishment of basic conservation method (active, passive, dynamic, static);
- identification and selection of populations to be conserved;
- definition of conservation objectives and specific targets;
- definition of management guidelines (if any); and
- Establishment of a monitoring system.

These activities present how such a complex tasks may be approached in a systematic way. It is far from being exhaustive and needs to be adapted to any given situation.

Table 3 shows the main parameters illustrating the above forms of tree species gene pool conservation *in situ*.

Table 3: Forms of gene pool conservation of tree species in situ in Serbia

Character of natural population	Number	Area
natural reserves	50	569.000 ha
		Fruška Gora
NATIONAL PARKS	5	Đerdap
		Tara
		Kopaonik
SEED STANDS	20	Šara
		Broadleaf species
		Coniferous species
INDIVIDUAL TREES	138	210 ha
		934 ha
	1122	

The great advantage of this form of in situ conservation is that the collection of reproductive material does not harm forest populations, or change the gene frequency. However, the desired objective of maintaining the genetic structure of the existing stands will not be achieved by factual application of in situ gene pool conservation strategy, thanks to normally occurring dynamic changes. If natural stands are left to "nature" and if genetic improvement measures are not implemented, the species composition and the distribution of gene complexes will change during the stand transformation from one stage of succession into another. Accordingly, the main obligation of in situ gene conservation is to tend the protected stands so as to maintain the desired genetic composition. However, this approach is generally either not understood or it is ignored. The measures which include protection, yield recording, and stand improvement cuttings should make the management of genetic reserves a normal and integral part of forestry practice. It is not necessary to preserve hundreds of thousands of individuals which contain the same desired gene complexes; maximally several hundreds of such trees are usually sufficient. If the individuals are protected and if their normal reproduction is ensured, there is no need to designate thousands and thousands of hectares for gene conservation.

The goal of the protected areas is ecosystem, spectacular natural beauty, or site protection for the animal species at risk. As they are as a rule protected by the law, the operational approach in them is passive, and the genetic approach is static. In protected areas and national parks, serious restriction is the legally defined availability of the genetic resources for breeding activities, and the predominance of old forests justifies the doubt in the safe regeneration and, accordingly, in the safe genetic conservation.

The existing number and the area of seed stands are insufficient and they do not reflect the richness of forest tree species gene pool of Serbia. Their decrease over the last decades cannot be justified either by the attained level of improvement study, or by market demands for wood. Natural forest populations, which are in forest terminology referred to as high normal forests, by their phenotype and population characteristics, represent the greatest accumulation of forest tree genetic resources.

Their area is sufficient and covers the widest possible genetic variability within the improvement zone, but it is smaller than the area of national parks. The main difference between these two categories is in the regeneration model. Planned regeneration, step by step, leads to the groups of tree genotypes which are more resistant to various harmful factors compared to the very old individuals in the national parks. As the environmental factors change in the long run, the younger parts of the population for genetic reserves will have a more adaptable potential, and the genetic resources of the regularly managed stands are more readily available and always at the disposal to the researchers. On the other hand, short-sighted exploitation or unfavourable impacts of environmental changes, including also land use changes, can soon reduce the genetic variation in natural populations managed for wood volume production and harvesting. The demands for valuable raw material expose the natural populations to a great economic pressure, especially when it is taken into account that modern forest exploitation technologies enable the cutting even on the previously inaccessible grounds. The inadequate regeneration can lead to the domination of another species in the area, or to the introduction of the provenances of unknown and undesired characteristics.

Gene pool conservation *ex situ*

The development of improvement programmes includes different methods of tree species *ex situ* gene conservation. The specificities of these methods are based on general biological characters of trees, among which the forms of reproduction - generative and vegetative are especially significant, and also the characters of changes in their populations and the potentials of seed banking.

Ex situ conservation in plants is usually applied in well-defined situations (FAO, 1989):

- to safeguard populations or individuals that are in danger of physical destruction when protection *in situ* is not possible;
- to safeguard populations which are in danger of genetic deterioration;
- to ensure a readily available, continuous supply of reproductive material, either by creating a production source or through storage;
- To allow commercial improvement of a species through breeding activities and supply of genetically improved reproductive material.

Ex situ populations or collections with forest trees are established and maintained for a number of objectives which most frequently can be classified into one or several of these four categories. Often *ex situ* conservation will be used as a complement to, or substitute for, *in situ* conservation of unique populations that are threatened in their natural habitat. The ideal approach of *in situ* conservation, or an integration of the two types of conservation methods, is often prohibited by

increased pressure on land, change in land use or by economic constraints. *Ex situ* conservation becomes very useful when it is desirable to establish a well-defined seed source for commercial plantations, without doing expensive breeding operations, and at the same time assure that the reproductive materials produced are well adapted to the ecological conditions in the forest.

There are many methods of conserving the desired genes or genotype complexes containing the genetic material which will ensure the directed use of the potentials of initial populations. Most frequently the goals are attained by reproduction, by conventional methods of generative reproduction by free or controlled crossing - outbreeding, inbreeding and uniparental reproduction and by vegetative reproduction methods - rooting, grafting and cell and tissue culture.

Any genetically sustained management of forest genetic resources within the framework of production forests, in situ or ex situ conservation must be based upon a sound knowledge of sexual reproduction. Sexual reproduction is the most important source of the genetic system to combine genetic variants, i.e. successful pollen production and egg formation, proper pollination, small amount consanguineous mating (or an early selection of consanguineous seedlings) is important cornerstones for a tree population to maintain to create genetic diversity. Although our knowledge is still very scanty as far as effective pollen and seed dispersal is concerned, forest geneticists probably have underrated the species capabilities of pollen distribution. Even in very low density forest stands there is seldom - if ever - a biologically significant increase of highly-inbred seedlings accounting for effective long distance dispersal and pre- and post zygotic mechanisms getting rid of inbreeds or avoiding them in the first place (GEBUREK, 2005).

The advantage of woody plants is that the selected genotypes can be conserved "for ever" by vegetative reproduction. It is no more necessary to save hundreds or thousands of hectares under trees, but by long-term testing, the tested ortets can be cloned into millions of copies, which is well illustrated by the experiences with the clonal varieties in the genera *Populus*, *Salix*, *Criptomeria*, etc. Some of the more modern methods, such as tissue culture, have a great potential in gene conservation ex situ. As this model is becoming increasingly operative, it will be possible to store the genetic potential of a high number of genotypes in a very small space.

Seed storing methods are very good for some species, as in this way genotypes, genes or gene complexes can be conserved. In the application of this method, the two important facts are that seeds lose germination energy over time and that the seeds have to be replaced by the new yield and that in some species seed aging is accompanied by gene mutation, so the resulting trees can have a different genetic component compared to the original population. Also, pollen can be stored for a long time, but pollen is only a half of the desired material, and the adequate female reproductive organs for pollination are also necessary. The more recent quantitative studies in population and ecological genetics point out that natural and domesticated tree species in Serbia contain significant pools of genetic variability,

both geographical and intrapopulation. There are many variants in each species, so the collection could include only a small part of the total variability. The basis of the use of initial material is the preliminary experimental research – the approbation of various populations in special geographical, ecological and population test plantations.

The results of *ex situ* gene pool conservation of tree species in Serbia have been achieved by the establishment of:

- provenance tests;
- clonal tests in a dense network of comparative clonal plantations and experimental-production macro experiments with numerous autochthonous and allochthonous clones of poplars, willows and black locusts;
- clonal and seedling seed orchards.

The above experimental populations were established to conserve the wide genetic variability for a long time for future improvement cycles, because they consist of the selected genotypes of tested origin. These populations were divided into lines, into major or key parts, or into small multiple populations. As the regeneration in them was planned by controlled crossing and progeny tests, also including selection, the number of unrelated individuals per generation was rather low and ranged between 69 and 500 in different models. The application of improvement methods is by all means effective for the set goals, but their advantages from the aspect of long-term gene pool conservation should be critically observed:

First, the material of which the experiments were established was always selected according to economically important characteristics, and the number of genotypes was relatively small;

Second, controlled crossing by any fertilisation design is more difficult to perform practically in the field than in the model, the expenditures could increase substantially, and the creation of safe conditions – prevention of contamination by undesired pollen – can be very complex;

Third, improvement lines will differ from the natural population.

Survey of the previous work on gene pool conservation of trees *ex situ* in Serbia

Provenance tests.—Provenance tests are applied in *ex situ* gene pool conservation of tree populations as one of the methods of assessing the degree of diversity and of the potentials of both autochthonous and allochthonous species. In addition to gene pool conservation, provenance tests:

- determine the potential and the degree of divergence of the isolated populations, in the sense of the higher adaptability and productivity;
- determine the relative differences in the quantities of genetic variability among the provenances, and the differences within and between the half-sib and full-sib lines in the same provenance.

In Serbia, provenance tests were established for numerous tree species and they were subject to intensive analyses: walnut /Tucović/, beech /Jovanović/, Austrian pine /Tucović, Isajev/, Norway spruce /Tucović, Isajev, Šijačić-Nikolić,

Ivetić/, Douglas-fir /Jovanović, Lavadinović/, Scots pine /Tošić/, wild cherry /Isajev, Ćurčić/ Balkan maple /Isajev, Ćurčić/, common oak /Erdeši/, etc.

The study of eight selected provenances of Norway spruce, *Picea abies* Karst, in repetitions at three sites near Ivanjica, at the altitudes of 600, 900 and 1200 m, included several morphometric and physiological parameters in the juvenile plant development (ISAJEV *et al.* 1992). This test included three Slovenian provenances - Menina, Mašun and Jelovica, and five Serbian provenances - Golija, Kopaonik, Zlatar, Radočelo and Čemerno (IVETIĆ 2004). The research was aimed at the study of Norway spruce differential characters and variability in a part of its natural range in South-Eastern Europe, the study of production potentials of individual provenances in more or less identical and different ecological conditions, and the collection of information on the characteristics and variability among and within the selected provenances at the experimental sites in Serbia. The results obtained to date confirm that the free genetic variability of Norway spruce is very high in all eight analysed provenances, and that according to the study results, Serbian provenances have a higher adaptability to very different ecological conditions. At the site of submontane beech, which is not within the Norway spruce natural range, spruce growth and adaptation were successful, which indicates that, in addition to the natural optimum in the zone of spruce belt *Picetum abietis serbicum*, its technogenic optimum can also be at the site of other species. The study results in all three altitudinal belts confirm the specificity of the Norway spruce gene pool in Serbia, the consequence of which is the fact that, in our regions, spruce has a special climatogenic belt, compared to other countries in the western part of the Balkan Peninsula.

Seed orchards.-The establishment of seed orchards specialised for multiannual production of improved seed material is simultaneously a significant contribution to conservation and testing of *ex situ* gene pools of individual tree species. In Serbia, the following clonal seed orchards were established: Austrian pine - 40 clones, Weymouth pine - 32 clones, Norway spruce - 30 clones, common oak - 50 clones, and seedling seed orchards: Serbian spruce - 5959 seedlings in 50 half-sib lines, Austrian pine - 5600 seedlings in 40 half-sib lines, Balkan maple - 2890 seedlings in 30 half-sib lines (ISAJEV *et al.* 1999).

The production of selected seeds in seed orchards is based on the number of incorporated genotypes and on free fluctuation of pollen, as the base of gene exchange of the incorporated trees. It is supposed that the seeds from seed orchards will inevitably have a narrower genetic base than the seeds from a "wild" stand. This potential is more certain for the characters such as slight taper and fullness of bole, cladoptosis, wood properties, or the angles of branch insertion, but not for physiological properties, such as survival, or the adaptation of seedlings cultivated from the seeds originating from the seed orchards. As the parents originate from many, very distant stands, the produced seeds will have a wide physiological diversity and the fear of poor growth or adaptation power due to the "narrower genetic base" is not reasonable. Such reduction in physiological adaptability is possible only if the desirable morphological and technological characters are strictly related to the adaptation characteristics of the progeny. The number of incorporated

genotypes in clonal seed orchards, from 15 to 30 genotypes is insufficient and gene exchange is often completely absent. This can be overcome by the transfer from clonal orchards to seedling seed orchards, established from the selected seedlings in half-sib lines of the corresponding plus trees.

The combining capacity of seedling seed orchards is considerably higher, and also a more successful gene exchange between the adjacent trees can be expected. This concept enables the compromise between contradictory demands for the maximal gene pool conservation on the one hand, and the tendency of enhancing the newly established plantations, on the other hand. There are numerous interactions between genotype numbers and the scope of gene exchange, which depend on the planting scheme and on the environmental conditions and the directions of selection already at the level of the gamete. Starting from the fact that the spatial layout of genotypes in the seed orchard determines the method of gene exchange between the adjacent trees in free pollination, the Serbian spruce seed orchard established with 5959 plants in 50 half-sib lines is based on the planting scheme with plants of the same line in one block. In this way, for the first time, the three basic types of Serbian spruce reproduction are ensured: inbreeding, outbreeding and uniparental reproduction. Inbreeding, which will be maximally induced by this planting scheme, is important from the following aspects: 1. Genetic research, 2. Process of further species improvement, and 3. Controlled production of genetically good-quality seeds and planting material. Depending on the category and genetic constitution, the seeds and planting material are:

- free pollination of 50 types of half-sib lines, intra line plants;
- 200 types of interlinear hybrids of family border rows,
- controlled pollination of 50 self-fertilised lines, 223 intraspecific hybrids and interspecific hybrids with Norway spruce, Sitka, Japanese, Serbian spruce, and other compatible species.

In addition to the trend of gene pool enrichment, the diversity of ecological factors requires the change in seed orchard spatial structure, consisting of several repeated more or less related orchards which should ensure higher yield, economy and stability of forest seed production. A seed orchard consists of several suborchards, representing a system of interconnected local entities, which more or less amortise the unfavourable environmental factors. The Austrian pine seed orchard at Jelova Gora near Užice (TUCOVIĆ *et al.* 1991), is established by this principle, as a contribution to the innovation of the seedling seed-orchards system. The layout and the same planting scheme within each, enables the regeneration of the parts which are destroyed or endangered. Different pollination treatments in the suborchard simultaneously represent the "experiments" which enable the experimental study of the seed tree potential, and the general and specific combine capacity. In subpopulations of tree plantations with xenogamous pollination, the percentage of trees with cross pollination is usually much lower than the suborchard proportions. The data on the pollen distribution and accumulation confirm that in seed orchards, there are no conditions for the panmixture. Ecological differences

among suborchards are the reality and they are very useful from the aspect of testing the incorporated genotypes as the promoters of orchard functionality in general.

CONCLUSION

Gene pool conservation of tree species includes a series of activities aiming at the sustainability and protection of genetic and species variability. This implies the investigation of genetic resources and their identification through the research of the genetic structure and the breeding system of individual species. Later on, this includes the study of intra- and inter-population variability in experiments - provenance tests, progeny tests, half- and full-sib lines, etc.

Ex situ improvement plantations offer the potentials that are absent or very rare in nature. By inbreeding, Serbian spruce seed trees form homozygous and heterozygous genotypes with detectable traits or gene markers which are rare in free pollinating populations. This fact, in combination with other methods, also enables their location within the Serbian spruce chromosome complexes. The recent methods of alloenzyme study, such as electrophoresis, can be applied even wider for the assessment of heterogeneity. Protection or conservation measures are crucial in the continuous supply of genetic variations. In the context of tree breeding, conservation measures are becoming increasingly important, because the progress in improvement will lead to the narrowing of genetic variability. If the wider genetic base is still available, the negative consequences caused by genetically uniform, but high-yield plantations, can be mitigated by the introduction of genes resistant to pests and diseases. It will probably be necessary to maintain a wide genetic base in the cases in which the goals of improvement programme are changed, and the new gene combinations are needed.

For many native forest tree species, we do not know their effective dispersal capabilities and we still must rely on rough estimates of pollen distribution based on pollen sedimentation velocities already investigated several decades ago. While paternity studies are undertaken for several forest tree species, in Serbia, such as Austrian pine, Serbian spruce, Norway spruce (ISAJEV *et al.* 2003, IVETIĆ 2004, LUČIĆ 2007) we still do not know the temporal pattern and spatial variation of gene flow and realize that important genetic building blocks are missing for a sound genetic management.

Efficient conservation of genetic variability depends on the total knowledge on the species, i.e. the occurrence and distribution, reproduction method, breeding system, genetic structure, and other related forms. In the absence of the sufficient study on individual species, which at the moment prevail in tree breeding programmes and in forest tree seed projects, the three important strategic approaches are as follows:

1. Implementation of the basic and generally accepted rules of sampling from the initial population, so as to establish the experiment, as well as the in-depth study of ecological characteristics of the site where the test or the tests are established;

2. Description and recording of the basic facts on the activities of identification and designation of the conservation area;

3. Application, enhancement and support to the study of the factors which affect the maintenance of genetic variability in reproduction biology and improved silvicultural systems.

The narrow relation between gene pool conservation, tree breeding and rational forest utilisation dictates the establishment of correct relationships between *in situ* conservation of forest genetic resources and sustainable forest management on the one hand, and protected area management, on the other hand, as well as the treatment, conservation and management of genetic resources *in situ* and *ex situ* and breeding activities as the complementary strategy. The focus of species diversity conservation should be transferred from the physical protection of stands or trees to the protection of their reproductive function. In this way, the potential genetic variability will be transformed into free genetic variability which is available by natural selection.

Each of the presented stages of protection and improvement has its specific purpose and in most cases it is only a part of total activities. The growing stock of National Parks and seed stands is rich in tree and shrub species, but its genetic variability is still insufficiently known, which creates the difficulties in its adequate utilisation in the establishment of new good-quality forest plantations. The shortage of specialised staff makes the issues of conservation and gene pool improvement even more complex. Therefore, the experimental solving of these problems is urgent in order to find the best method of tree gene pool conservation and improvement *in situ* and *ex situ*. The forests of National Parks, seed stands, reserves, live archives, arboretums, provenance tests and seed orchards are much more significant than it was shown by the previous active attitude to them.

The study of population characteristics and the effects of natural and experimental selection on the genetic structure of forest tree populations contribute to the conservation and enhancement of the gene pool of autochthonous woody plants. The balance model of the population genetic structure reveals the new traits and requires further research.

The increased use of the genetic potential in tree improvement in Serbia should be intensified by the following activities: improvement of production of normal forest seed, application of the concept of new selections directed primarily to the improvement of only one character, because in that case the result would be certain, establishment and management of seed orchards as specialised plantations for long-term production of genetically good-quality forest seeds, and the shortening of the improvement process by introducing new techniques and methods (molecular markers, somaclonal variation, genetic engineering, protoplast fusion, micropropagation, etc.).

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ZASTITA GENOFONDA I OPLMENJIVANJE DRVEĆA U SRBIJI

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I z v o d

Zaštita, testiranje i korišćenje genofonda vrsta drveća, u Srbiji obuhvata više sukcesivnih aktivnosti *in situ* i *ex situ*: a) izučavanje prirode fenotipske varijabilnosti u velikim i malim populacijama, b) unapređenje tehnike masovne i individualne selekcije, c) primenu bliske i udaljene hibridizacije; d) analizu morfometrijskih karakteristika, e) upoznavanje međuzavisnosti osobina rasta i razvoja analiziranih genotipova i njihovog potomstva (Isajev, V. et all 1988). Aktivnosti na očuvanju i korišćenju genofonda, podrazumevaju njegovo očuvanje *ex situ*- putem reprodukcije šumskih populacija i superiornih genotipova, osnivanjem specijalizovanih objekata, arboretuma, živih arhiva, provenijeničnih testova, testova potomstva, klonskih testova i semenskih plantaža. U radu su predstavljeni oblici i značaj očuvanja genetičkog diverziteta populacija drveća primenom metoda *in situ* i *ex situ* konzervacije genetički resursa šumskog drveća. U cilju upoznavanja, *ex situ* očuvanja i usmerenog korišćenja genofonda, omorike, smrče, crnog bora i planinskog javora u Srbiji su osnivani specijalizovani pilot objekti. Rezultati genetskog vrednovanja smrče u provenijeničnom testu, omorike, crnog bora i planinskog javora u generativnim semenskim plantažama su savremen pristup u čuvanju i testiranju njihovog genofonda kao i za planiranje i podizanje budućih kulturnih zajednica ovih vrsta.

Dobijeni rezultati su opsnova za bolje upoznavanje proizvodnog i adaptivnog potencijala analiziranih vrsta. Semenske plantaže i pilot objekti, kao specijalizovane kulture, treba da doprinesu ne samo prevođenju potencijalne genetičke promenljivosti u slobodnu, kao osnove usmerenog korišćenja genofonda drveća, već i kao poligoni za testiranje i očuvanje biodiverziteta vrsta.

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