Annals of Silvicultural Research
38 (2), 2014: 51-61

http://ojs-cra.cilea.it/index.php/asr

Conference paper

Forest genetic resources to support global bioeconomy

Saša Orlović[1*], Mladen Ivanković[2], Vlatko Andonoski[3], Srdjan Stojnić[1], Vasilije Isajev[4]

Received 30/09/2014 - Accepted 24/11/2014

Abstract - A biobased economy implies sustainable and effective use of the biomass. This includes new products from forestry. The sustainable production, use, consumption and waste management of biomass all contribute to a bioeconomy (The European Bioeconomy in 2030).

In the context of bioeconomy the conservation of forest genetic resources assumes a key significance in overcoming global challenges such as climate change. Forests are expected to play a key role in climate change mitigation, but they will only be able to fulfil that role if the trees themselves are able to survive and adapt to changing climate conditions. Genetic diversity provides the fundamental basis for the evolution of forest tree species and for their adaptation to change. The enormous range of goods and services provided by trees and forests is both a function of and testimony to the genetic variability contained within them. Conserving forest genetic resources is therefore vital, as they constitute a unique and irreplaceable resource for the future, including for sustainable economic growth and progress and environmental adaption (The State of the Worlds Forest Genetic Resources 2014). Previous research of population characteristics and the effects of natural and artificial selection on the genetic structure of populations contribute to the conservation and enhancement of the gene pool of the native tree species. The balance model of the population genetic structure reveals the new properties of the populations and requires further investigations, especially of the relations of subpopulations, half-sib families and organisms and the effect of variable factors of the environment, on the exchange of genetic material within natural and cultural populations.

Being of national and international significance, these resources require intensive protection and enhancement *in situ* and *ex situ*. In this paper a general introduction is given to conservation of forest genetic resources in Serbia, Croatia and Macedonia in the context of bioeconomy. Based on the current situation of conservation of forest genetic resources, some strategic suggestions concerning the future development of genetic conservation is given, taking into consideration the conservation objectives and future trends of great impact on existing forest genetic resources.

Keywords - Genetics resources, conservation, bioeconomy

Introduction

The concept of “bioeconomy” or “bio-based society” has become an important component of national, EU and global policies. The social, economic and biological challenges we face, and the scarcity of natural resources combined with climatological changes, necessitate new approaches to knowledge and innovation as well as to knowledge-based policies. The transformation to a bio-based economy means a transition from a fossil fuel-based economy to a more resource-efficient economy based on renewable materials produced through sustainable use of ecosystem services from land and water. A greater focus on research and innovation can provide us with new products developed from biomass that will replace fossil material, combat climate change, reduce waste and create new jobs.

A bio-based economy (bioeconomy) can be defined as an economy based on (The European Bio-economy in 2030):

a) The sustainable production of biomass to enhance the use of biomass products within a number of different sectors of society. The objective is to reduce climate effects and the use of fossil-based raw materials.

b) Increased added value for biomass materials, concomitant with a reduction in energy consumption and recovery of nutrients and energy as additional end-products. The objective is to optimise the value and contribution of ecosystem services to the economy.

Climate change influences both the forest as an ecosystem and also sustainable wood production. The forests need to be adapted to climate change, to continue to secure both their function for use, protection and recreation, and also the role that wood and the forest play in protecting the climate (The State of the Worlds Forest Genetic Resources 2014).

An important objective of the conservation of genetic resources is to maintain the adaptedness of organisms to changing environmental conditions. By conserving sufficient amounts of heritable variation in different species and thus their evolutionary

[1] University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad, Serbia
[2] Croatian Forest Research Institute, Jastrebarsko, Croatia
[3] University Ss. Cyril and Methodius - Faculty of Forestry, Skopje, Macedonia
[4] University of Belgrade, Faculty of Forestry, Belgrade, Serbia
* corresponding author: sasao@uns.ac.rs

http://dx.doi.org/10.12899/ASR-942

potentials, life can continue under changing and even new conditions. The possibilities for future generations to meet their varying demands are thus secured (Rajora and Mosseler 2001).

The genetic variation of most agricultural and horticultural crops as well as of farm animals can be collected and conserved in so-called gene banks. However, forest genetic resources are usually conserved as living trees in growing forests.

Considering the objective of preserving the broadest genetic diversity, not only the most representative trees populations or important single trees should be subject to gene conservation. Populations from marginal localities also need to be conserved, despite their lower economic importance as such populations and trees may carry genes of importance for breeding (adaptability, resistance). *In situ* and *ex situ* measures are necessary to complement each other (Andonovski and Velkovski 2011).

Conservation, testing and utilization of tree species gene pool involves several successive *in situ* and *ex situ* activities such as: a) study of the nature of phenotypic variability in large and small populations, b) improvement of mass and individual selection techniques, c) application of intervarietal and distant hybridization, d) analysis of morphometric characters, e) familiarity with the correlation of growth characteristics and the development of the analyzed genotypes and their progeny (Isajev et al. 1988). Activities contributing to gene pool conservation and utilization imply its conservation *ex situ* - through the reproduction of forest populations and superior genotypes by establishing specialized seed sources, arboretums, live archives, provenance tests, progeny tests, clonal tests and seed orchards (Gustafsson 1950, Jovanović 1972, Isajev et al. 1995, Skrøppa 2005). *Ex situ* populations of forest trees in Serbia, Croatia and Macedonia are established in the aim of protection and directed utilization of the gene pool of physically endangered populations or individuals, as a supporting activity to conservation *in situ* and for the provision of readily available constant supplies of genetically improved reproductive material.

The paper presents preliminary results of multiannual analyses which are being carried out in specialized plantations of different tree species aimed at testing of genotypes of parent individuals as well as their half-sib lines. The results of multiannual analyses lead to a better knowledge of the production and adaptation potentials of analyzed species. Seed orchards and pilot plots, being the specialized plantations, should contribute not only to the conversion of the potential genetic variability into free one, as the base of directed utilization of tree gene pool, but also they are the polygons for testing and conservation of species biodiversity.

Conservation of genetic resources *in situ* in Serbia

In order to conserve gene pool *in situ*, in Serbia there are six National Parks, ten Regional Parks, 50 Reserves and 158 Seed Stands of major economic species of broadleaves and conifers. National Parks are: Fruska Gora – 25’300 ha, Djerdap – 63’608 ha, Tara – 19’200 ha, Kopaonik – 11’810 ha, and Shara – 39’000 ha (total 158’918 ha). In the National Parks Fruska Gora and Djerdap, mostly the communities of deciduous tree and shrub species are represented, in the Parks Tara, Kopaonik and Shara, the communities of coniferous species are predominant. Seed stands, along with the production of good quality seed stock, are also intended for the conservation of the gene pool of tree and shrub species in situ, as they contain plus and normal trees and the trees which represent the average of the population. Seed stands are listed in the Proposed Register of Forest Seed Sources in Serbia, with separate lists of broadleaf and coniferous species. The Register includes 151 coniferous seed sources - total area 1’476.1 ha, of which 36.9 % are fir, 28.5 % spruce, 14.2 % Scots pine, 11.8 % Austrian pine, 4.1 % Serbian spruce. There are 65 seed stands of broadleaf species covering 1’665.2 ha, of which 30.5 % are Sessile oak stands, 31.1 % Pendunculate oak, 17.1 % Common beech, 21 % Turkish hazel, 8.4 % lime and 2 % other species. The Registers of seed stands were made twenty years ago, so on the occasion of their revision, only the best populations, i.e. the best trees should be chosen by which the greatest possible portion of the desirable intraspecies variability will be included. Also the work should be intensified on the study and zonation of seed utilization from the zones containing seed stands of major economic species of trees.

Conservation of genetic resources *ex situ* in Serbia

The opportunities for the singling out of Nature Reserves, and in this respect, the protection of forest natural resources, are proportionally limited. For these reasons, Norway spruce provenance test and specialized sources and plantations have been established in Serbia to conserve the genetic resources of woody species *ex situ*, by the reproduction of selected species in forest populations. They are: (a) seed orchards, (c) provenance tests, and (d) progeny tests. Each of the above categories has a special purpose, which is coordinated with the needs of gene pool protection and enhancement.

Seedling seed orchards were established from 50 Serbian spruce half-sib families on the area of 2.7 ha (Isajev et al. 1990), Austrian pine - 40 families on 3 ha (Tucović and Isajev, 1991), divulge wild cherry - 30 families on 1.5 ha, Pedunculate Oak – 76 ramets (Erdeši 1996, Orlović et al. 1999 and 2002), Pedunculate Oak – 122 families (Orlović et al. 2001). The yield in these plantations has not yet reached the level of commercial exploitation. However, the progeny from seedling seed orchards will be more variable than that from clonal ones, and consequently seedling seed orchards are better for the purpose of gene pool conservation.

Norway spruce provenance test in Serbia

Morphological variability and changeability of physiological properties of intraspecial taxons of Norway spruce are described, among other researches, on the bases of provenance tests in Europe and North America (Lines 1967, Kleinschmit 1970, König 2005). The results obtained in the analyses of these tests enabled better familiarity with the production and adaptive potential of the Norway spruce gene pool and the ecological factors which determine the range of its horizontal and vertical distribution were established (Isajev et al. 1990).

For the establishment of a provenance test in the vicinity of Ivanjica 3 locations with different altitude, exposition and area as well as different site characteristics were chosen (Isajev et al. 1992). In the test plantations, five Norway spruce provenances from Serbia are comparatively examined: Golija, Zlatar, Cemerno, Radocelo and Kopaonik, as well as three Slovenian provenances - Jelovica, Menina and Masun. On the basis of data of the Hydro meteorological service in Belgrade regarding the area of Ivanjica, climatic conditions of all three locations were studied. In the very areas in which the test plantations were established topographic surveys were carried out and a total of 8 soil profiles were opened.

The first test plantation is in the department 51, at an altitude of 570 to 610 m, northern exposition and the total area of 2.02 ha, on deep acid soil (dystric cambisol) and the geological base were schists. The second test plantation with the area of 0.65 ha is in the department 38 of FMU Kovilje- Rabrovica: south-eastern exposition, altitude of 1’105 to 1’125 m on dystric cambisol on schists. The third test plantation was established in the department 46, section C in FMU Golija at an altitude of 1’560 to 1’570 m. The exposition was north-eastern and the area was 0.73 ha, the soil was podzolic brown. The number of Norway spruce seedlings planted in test plantations was 2’442.

The results of prior analyses show that the impact of natural selection is the most distinguished in field experiments realized at three altitudinal levels, in which Serbian provenances demonstrate greater adaptability to very different ecological condition (Ivetić 2004). It was recorded that even in sites of submontane beech which is not within the natural range of spruce its growth and adaptability were successful which proves that besides its natural optimum in the zone of spruce belt *Picetum abietis serbicum* s.l., its technogenic optimum can also be reached in sites of other species. The results obtained from all three altitudinal belts contribute to the explanation why Norway spruce in Serbia has a specific climatogenous belt compared to other countries of the Balkan Peninsula. From the expert perspective prior researches play an important role in the economy because they facilitate the choice of a provenance, or a group of provenances, suitable for certain sites as part of planning of afforestation and reclamation works in degraded stands and sites.

Austrian pine seed orchard in Serbia

Large areas of bare land and degraded sites which require urgent afforestation as well as the capability of Austrian pine to achieve good results in extremely bad sites, set forth the need for organized seed production and seed forests as priority aims. In the aim of fulfilling these needs adequately, there is a need for intensified scientific and expert activities on the establishment and management of Austrian pine seed orchards.

Generative seed orchard of Austrian pine in Jelova Gora, with the area of 2.70 ha on the site *Fagetum montanum* Rud. was established in 1991 from 5’422 two-year-old seedlings in 40 half-sib lines of the test trees selected in seed forests Sargan-Mokra Gora and Crni Vrh-Priboj (Fig. 1) (Isajev et al. 1992). Austrian pine seed orchard with 40 half-sib lines, with three repetitions each, in each of the five subplantations and dynamic environmental factors-altitudinal difference of 20 m, two expositions and two soil types is the first generative seed orchard of metapopulation structure established in Serbia. Its structure enables the realization of genetic and development mechanisms and mechanisms of regulation on the one hand and realization of the effects of ecological mechanisms on the other. The above mentioned will benefit gene pool conservation of this species.

Multi-annual research involved detailed study of the variability of nine morphological seedling parameters - seedling height, annual height increment, root-collar diameter, diameter of horizontal crown projection and the number of branches in the

Figure 1 - Generative seed orchard of Austrian pine in Jelova Gora.

last three whorls. The obtained data revealed great variability even in case of slight site changes, as well as great adaptability of the incorporated planting stock. Analysis of correlation confirmed significant positive correlation of almost all elements of growth.

The research of qualitative characteristics also illustrates great genetic diversity. The following was observed: flowering at the age of five years, seedlings with grey needles, three-needle pines, smaller sized needles, proliferation.

The investigation of variability of the root system of Austrian pine seedlings created a base for the selection of genotypes with favourable characteristics in the sense of better adaptation to shock after transplanting or growing in extremely arid stands.

The analyses of the participation of photosynthetically active pigments - chlorophyll a and b and carotenoids are among the first analyses of that kind carried out regarding conifers in this country. These researches contribute to the improvement of familiarity with the correlation of the analyzed photosynthetic matters and basic elements of growth. Prior research of the variability of juvenile Austrian pine and the correlation of morphological and physiological parameters have their multiple importance for science as well as for practical application, being the base for the improvement of the good quality seed and planting stock with favourable characteristics. On the basis of one-way analysis of variance of all the examined morphometric properties it was concluded that significant differences appear at the inter-line and inter-provenance level which indicates that the differences among half-sib lines and provenances are not the consequence of random errors i.e. random variations. By using an LSD - test groups of half-sib lines according to the years of research were homogenized, which confirmed statistically significant differences and the superiority and inferiority of certain lines, previously determined on the basis of relative percentage of average heights and half-sib line diameters in average values of certain provenances. Connecting of clusters of half-sib lines with the highest and the lowest average values of all the examined quantitative parameters within the seed forest (1) is at a shorter total distance than in the seed forest (2) on the basis of which it is also confirmed that the sample of seed forest (1) shows higher homogeneity and lower intra-population variety. The applied statistical analysis indicates great genetic diversity within the seed orchard, but clear differences between certain half-sib lines cannot be strictly determined because that would require full-sib progeny tests.

The obtained results are important for the directed utilization of the gene pool of this species and as directions for the improvement of techniques used in the establishment of Austrian pine seed orchards of the second, third and later generations.

Serbian spruce seed orchard

On the basis of the applied analyses of multi-

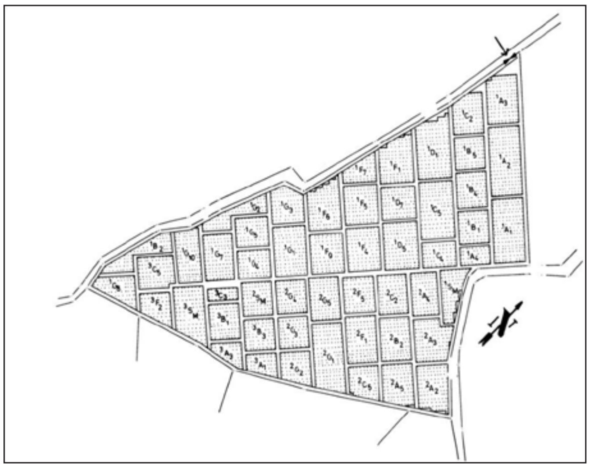

Figure 2 - Generative seed orchards of Serbian spruce (*Picea omorika*/Panć/Purkyne).

annual research of the genetic variability of Serbian spruce (Isajev 1987), generative Serbian spruce seed orchard was established in Western Serbia in 1987 (village Godovik, near Pozega), on an area of 2.70 ha. As much as 5'959 seedlings (age 2+3) were incorporated into the seed orchard. The seedlings from the same family were planted in the same block, with random distribution in the form of a square-shaped planting scheme 2 x 2 m (Fig. 2).

The applied selection and hybridization produced reliable data on general and specific values of half-sib lines and incorporated genotypes. In this seed orchard, based on the planting scheme in which genotypes of the same line are in one block, we made possible, for the first time simultaneously, the three basic types of Serbian spruce reproduction: inbreeding, outbreeding and uniparetal. The study data are a base for further work on the directed utilization of Serbian spruce genetic potential.

By multiannual analyses of individual and line variability of a great number of continuous and discontinuous vegetative and reproductive characters, parental genotypes were selected in order to direct seed crop parameters by spontaneous and controlled hybridization. The study of several flowering parameters, intra- and inter- half-sib lines, such as abundance and regularity of micro- and macrostrobile formation, differentiation of genotypes into functionally male, i.e. female ones, analysis of pollen quality and seedling analysis, resulted in valuable information necessary for successful hybridization. After controlled hybridization performed by the model of incomplete diallel cross, which included 48 different parental combinations, we studied a part of genotype structure of parent individuals and their hybrid combinations, based on the analysis in the salts of soluble proteins of seeds, as the most common polymorph markers at the level of gene products. In these analyses, we used the seed from free pollination of half-sib lines, which were functionally female i.e., male, and the seed of parental genotypes and their hybrid combinations. In parental genotypes and their hybrid combinations, the obtained electrophoregrams were used for the calculation of the coefficient of similarity, at the levels mother-hybrid, father-hybrid and mother-father. Electrophoregram analysis of hybrid combinations shows the existence of different types of protein fractions - bands: bands common to both parents, bands originating one from father, one from mother (codominance of parent gene expression in the hybrid), bands originating from mother only, bands originating from father only, and bands specific for the hybrid.

Based on the results of multiannual study, we differentiated such hybrid combinations which show the highest mean values of the analyzed morphometric characters of the cones, i.e. seed crop, as well as better parental genotypes which show good general and specific combining ability. The obtained data were the basis for the construction of the model of experimental clonal seed orchard for the production of Serbian spruce intraspecific hybrids.

Balkan maple seed orchard in Serbia

Forest Estate "Golija" from Ivanjica established a Balkan maple (*Acer heldeichii*) seed orchard in 1994 (Isajev 1994). The location of the orchard is on the site *as. Fagetum montanum Rud.s* in the forest management unit "Kovilje-Rabrovica", department 12. The altitude of the location is 950 to 1020 m and the exposition is north-eastern. The area of the seed orchard is 1.05 ha (Ćurčić 1997 and 1999). The planting stock was produced in the forest nursery in Ivanjica from the seeds of 26 seed trees, selected in Golija, which had above-average morphologic (technical) and physiological (abundance and regularity of seed yield) characteristics in the population (Isajev et al. 1994).

Planting of seedlings of the age 2+0 was carried out in the spring of 1994. Distribution of planting was planned and realized in blocks. There are 6 blocks in total, 4 of which have a regular rectangular shape and 2 are in the shape of a scalene triangle. The irregular shape of the blocks depends on the shape of the area determined for seed orchard establishment. The realized distribution of the seedlings in blocks is based on the so-called metapopulation strategy of the establishment of generative seed orchards of forest trees (Tucović and Isajev 1991).

Planting of 2'962 seedlings from 26 half-sib families created the base for further works on the testing and becoming familiar with the gene pool of the populations in which mother trees were selected, as well as for the improvement of this interesting and precious species of our valuable broadleaves.

After the application of the appropriate tending, good quality seed for further reproduction will be produced. On the basis of multiple analysis of the genetic value of the incorporated genotypes, decisions on further works on the improvement of Balkan maple and the establishment of seed orchards of future generations will be made.

Taking into account the advantages of the lower, warmer locations for the establishment of seed orchards due to their favourable effect regarding the abundance and frequency of flowering and seed yield and the applied method of metapopulation structure, it can be expected with certainty that the established generative seed orchard of Balkan maple near Ivanjica achieves the expected production of good quality seed.

Pedunculate oak seed orchards in Serbia

There are two seed orchards of Pedunculate oak (*Quercus robur* L.) in Serbia – clonal and generative. They are established at the territories of Forest Units “Morović” and “Klenak”, which belong to the Public Enterprise “Vojvodinašume”, Forest Estate “Sremska Mitrovica”.

Clonal seed orchard was established in the period between 1979 – 1983 (Erdeši 1996). It is established of phenotypically superior genotypes from natural populations. The main criteria for selection of stems were the straightness of stem, branching, fast growth and resistance to oak powdery mildew (*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.). The seed orchard was established at the area of 7 ha, from 86 genotypes, which were multiplied by grafting into 2’520 remets. Depending on the scion thickness, five techniques of grafting were applied: simple copulation, English copulation, “mjesok”– little sack, cutting and “goat leg”. The seed orchard is composed of four varieties indigenous in the valley of the Sava river: early pedunculate oak (*Q. robur* var. *praecox*), typical pedunculate oak (*Q. robur* var. *typica*) and two varieties of late pedunculate oak (*Q. robur* var. *tardiflora* and *Q. robur* var. *tardissima*) (Orlović et al. 1999, 2001 and 2002). Clonal seed orcharad has been an object of numerous researches focused on genetic variability of acorn and leaf morphological and anatomical characteristics (Nikolić and Orlović 2002, Nikolić et al. 2003, 2005, 2006 and 2010). Likewise to the previously mentioned researches, in order to give the first insights into the genetic structure and diversity in the clonal seed stand, the aim of recently conducted study was to characterise the genetic structure related to different phenology of sampled oak genotypes using a system of established microsatellite molecular markers. Leaves from fifteen individuals were sampled from four different varieties of pedunculate oak (*praecox*, *typica*, *tardiflora* and *tardissima*). Seven microsatellite primer sets were used designed to be specific to the sequences flanking the $(GA/CT)_n$ and $(AG/TC)_n$ dinucleotide repeat motives in oak genome. *Quercus* species have revealed high levels of polymorphism suggested that these markers are well suited for studies of genetic diversity within oak population and between different varieties. Successful amplification of all observed microsatellite loci revealed allelic polymorphism between and within all varieties established the variety specific genetic structure (Galović et al. 2014).

Generative seed orchard is founded in the period between 2000 – 2004 at the area of 10 ha. It is established from the acorn that was sown. Acorn is collected from clonal seed orchard previously mentioned and phenotypically superior genotypes from natural populations. Orchard is composed from 129 families, in different number of replication (min. six replications), so the total number of plants (genotypes) in orchard amount 2’585. The space between trees is 7 x 5 m. Similarly to clonal seed orchard, all for varieties indigenous in the valley of the Sava river is represented in the generative orchard. Researches in the orchard have been started recently, collection of acorn from various families and establishing of progeny test.

European beech provenance trials in Serbia

European beech (*Fagus sylvatica* L.) provenance trials in Serbia were established in the spring of 2007. One of the trials is situated on the territory of National park "Fruška Gora" (Northern Serbia), while the second one is located on the territory of the Scientific Centre of the University of Belgrade, Faculty of Forestry – "Majdanpečka Domena", in Debeli Lug (Eastern Serbia) (Stojnić et al. 2012a). The trials are founded within the European network of beech provenance trials. On that occasion, in order to study the genetic variation relevant for adaptation among provenances in the Balkan region, 20 provenances of Croatia, Serbia and Bosnia and additional 12 for comparison from Austria, Germany, Hungary, Italy, Switzerland, and Romania were planted, of which 15 provenances are common to all trials. A total of seven provenance trials were established in Bosnia and Herzegovina, Croatia, Serbia (2), Germany (2) and Italy (von Wuehlisch et al. 2010). The main objectives in these trials could be arranged into four groups: 1) tree improvement, 2) gene conservation, 3) evolution biology and 4) stimulation of European co-operation in forest research (von Wuehlisch 2004).

Previous studies in the provenance trials in Serbia have been focused mainly on the examination of genetic variation within and among different provenances, as well as research of phenotypic plasticity. The aforementioned studies have included numerous parameters that could be, roughly, classified into: physiological, biochemical, morphological, and the parameters of the anatomy of wood and leaves. The research results indicate the existence of significant genetic variation both within and between different provenances, as well as ecotypic pattern of genetic variation (Stojnić et al. 2010, 2012b and 2013, Štajner et al. 2013). Also, given that some authors believe that in order to improve the adaptability of the population, special attention should be paid to phenotypic plasticity, as an alternative to genetic adaptability (Šijačić-Nikolić and Milovanović 2010), attention has been devoted to the study of phenotypic plasticity of wood anatomical structure. The results showed an existence of a plastic response of provenances, as well as the ability of provenances originating from moist sites to adapt to drier habitat conditions (Stojnić et al. 2013).

In situ conservation of forest genetic resources in Macedonia

In Macedonia, *in situ* gene conservation is mainly achieved through the establishment of protected areas and so-called gene reserve forests. In addition to these there are also long-term genetic studies and breeding populations (Andonovski 1995).

National parks and nature protected areas are of great importance for maintaining or improving the forest genetic resources. These areas in Macedonia are classified as follows (Andonoski 2011):

A comprehensive resource inventory on the nature protected areas was set up and management regulations were established for the natural reserves.

Table 1 - National parks and nature protected areas (coniferous species).

Name	Area (ha)	Description
National park "Pelister"	12'500.0	The best preserved natural stand of Macedonian pine (*Pinus peuce*)
National park "Mavrovo"	73'088.0	Natural stand of fir and spruce (*Abies borisii-regis, Picea abies*)
Nature reserve "Rozden"	3.5	Crimean pine (*Pinus nigra ssp. pallasiana*)
Nature reserve "Tumba"	5.0	Fir (*Abies borisii-regis*)
Nature reserve "Golem Kozjak"	4.0	Scots pine (*Pinus silvestris*)
Nature reserve "Popova sapka"	5.2	Norway spruce (*Picea abies*)
Nature reserve "Rupa"	7.6	Fir (*Abies borisii-regis*)
Natural reserve "Tsam Tsiflik"	490.0	Crimean pine (*Pinus nigra ssp. pallasiana*)
Natural reserve "Rutsica"	1'785.0	Dwarf mugo pine (*Pinus mugo var. mughus*)

According to the Law on Forests, selected natural seed stands for production of seeds belong to category of forests with special purposes and they are under special management regime. During the latest period, an increased effort to conserve and enhance the forest genetic resources has been undertaken on the basis of present knowledge about variability and heritability.

The first mass and individual selection in Macedonia was performed in 1962-1965 and the following coniferous seed stands of were selected and registered (Andonoski 1994):

Table 2 - Selected seed stands (conifers).

Species	Area (ha) Total	Area (ha) Reduced	Age (years)	Provenance
Abies borisii-regis	182.9	84.4	81	Indigenous
Pinus nigra ssp. pallasiana	258.9	137.5	73	Indigenous
Pinus silverstris	45.5	32.8	80	Indigenous
Pinus peuce	5.0	3.4	95	Indigenous
Pseudotsuga menziesii	2.8	2.1	35	Exotic

In 2008, new program for conservation of forest genetic resources in Macedonia started with the revision of the current seed stands of various economically important native and exotic tree species. This program includes preregistration of the current seed stands and seed orchards and registration of new, including those of broadleaved species. Following is the table of registered seed stands under the latest Law on forest reproductive material:

Table 3 - Registered seed stands under the latest Law on forest reproductive material.

Species	Number of seed stands	Area (ha)
Pinus nigra ssp. *Pallasiana* (native)	8	218.7
Pinus sylvestris (native)	7	131.8
Abies borisii regis (native)	16	375.9
Pinus peuce (native)	3	68.3
Larix decidua (exotic)	3	34.9
Pseudotsuga menziesii (exotic)	9	59.2
Sequoiadendron giganteum (exotic)	1	4.4
Robinia pseudoacacia (exotic)	1	1.2
Fagus moesiaca (native)	7	326.1
Quercus petraea (native)	2	35.2
Total	**57**	**1'255.7**

In situ forest genetic conservation in Macedonia includes the "dynamic" approach which encourages the adaptation of forest trees to the changing environment through naturally occurring evolutionary processes. This can maximize adaptability with the sufficient number of trees in the genetic resource population (Andonoski 1974).

Ex situ conservation of forest genetic resources in Macedonia

Ex situ conservation of forest genetic resources in Macedonia includes establishment of *ex situ* gene conservation stands, seed orchards, clone archives or individual trees. Conservation of individual coniferous tree species was carried out using "plus" trees selected for the development of tree improvement programs. The following "plus" trees were selected (Andonoski 1988):

Abies borisii-regis	42 "plus" trees
Pinus silvestris	62 "plus" trees
Pinus nigra ssp. pallasiana	82 "plus" trees
Pinus peuce	20 "plus" trees

On the basis of these selected "plus" trees the following Seed orchards were established:

Table 4 - Seed orchards (conifers).

Species	Type	Year of rising	Area (ha)	Fructification
Pinus peuce	clonal	1963	1.1	full
Abies borisii-regis	clonal	1963	0.5	full
Pinus silvestris	clonal	1978-1980	2.5	full
Pinus silvestris	generative	1978	5.0	full
Pinus nigra ssp. *pallasiana*	clonal	1988	1.5	started

Outlook

In the past more emphasis was placed on the conservation and study of "plus" trees, so now it is necessary to focus on study and conservation of the most valuable populations. The majority of gene reserves were selected in the early 1960, thus it needs repeated inventory with biochemical, cytological and molecular genetic methods.

It is necessary to get more information about genetic structure and differentiation of the tree species.

In situ conservation activities should be integrated part of the regular forest management. The major challenges for the conservation of forest genetic resources in Macedonia include population decline and population structure changes due to forest removal and conversion of forest land to other uses, forest fragmentation, forestry practices, climate change, disease conditions, introduced pests, atmospheric pollution, and introgressive hybridization. Developing scientifically sound conservation strategies, maintaining minimum viable population sizes, and deployment of genetically engineered organisms represent other important challenges in conservation. Both *in situ* and *ex situ* forest genetic resource conservation strategies must include the use of various biochemical and molecular genetic markers, adaptive traits, and genetic diversity measures. So, major opportunities for conservation of forest genetic resources in Macedonia include: use of molecular genetic markers and adaptive traits for developing conservation strategies; *in situ* conservation through natural reserves, protected areas, and sustainable forest management practices; *ex situ* conservation through germplasm banks, common garden archives, seed banks, DNA banks, and tissue culture and cryopreservation; incorporation of disease, pest, and stress tolerance traits through genetic transformation; plantation forestry; and ecological restoration of rare or declining tree species and populations.

Conservation of forest genetic resources in Croatia

Croatia, with its area of forest and forest land (2.49 million hectares, which is 44% of mainland Croatia) has 260 indigenous wood species. 50% belongs to forest ecosystem and 60 of them make economical richness of Croatian forests while there is more than 100 species which are added to the forest ecosystem to implement their biodiversity. Conservation of genetic diversity of our forest species represents the foundation of a sustainable forest management and preservation of natural structure of our forest stands, currently making 95% of the total woodland area. Croatia's richness in diversity of geographical regions has resulted in various ecological types and a large number of forest trees that are directly affected by habitat degradation, different types of soil, air and water pollution, excessive use of some more valuable species of forest trees, increasing impact of global climatic changes, as well as by anthropogenic effects (Kajba et al. 2006).

The need for conservation of genetic variability is related to the species pertaining to social broadleaves which are economically the most prevailing species (Pedunculate oak, Sessile oak, and Common beech). Among the conifers, Silver fir (*Abies alba*) is the most endangered species, with more than 70% of its population being permanently damaged. Other native coniferous species must be preserved from the deprivation of genetic variability as well.

Conservation of noble broadleaves should encompass a larger number of species from various genuses (*Fraxinus*, *Alnus*, *Ulmus*, *Prunus*, *Juglans*, *Castanea*, *Sorbus*, *Acer*, *Malus*, *Pyrus*, *Tilia*). They are partially endangered because of their exposure to different diseases and pests, as well as by continuous exploration caused by their technical value. Changes in hydrological conditions of our rivers have generated difficulties in restoration of riparian forests, and decreased genetic variability of European black and white poplar in their habitats. In coastal areas of our country, there is a need for conservation of genetic resources of Dalmatian black pine (*P. nigra* ssp. *dalmatica*) and our Mediterranean oaks.

Genetic diversity conservation of various species of forest trees is conducted within the programs that include in situ dynamic methods and ex situ static methods.

Conservation of native species within in situ method is based on concept of status quo of natural conditions protections on local environment, where is optimal alleles frequency to survive and reproduction in that environment reached. Starting point is that local population of certain species are resistant and adapted to environment stress, diseases and injurers impact. Conservation of genetic diversity researches contains knowledge about the smallest population size (MVP) which is required for their relative safe survival according to genetic, demographic, environmental and other factors. According to the size and type of areal of each species (continuous and discontinuous areal, genetic drift, etc.), we need to define number of subpopulation and units which will successfully present, include and conserve complete variability of each species. Conservation within *in situ* method differentiate populations in categories of protected objects of biological and landscape diversity, natural forest stand and population which already are or will be excluded from regular management (e.g. seed stands).

Protection by *ex situ* method represents forest tree species conservation out of their natural habitat. This method is used parallel to *in situ* method, especially with species where conservation of population or their parts is not possible. For that cause, setting of experimental surfaces with *ex situ* methods is required and includes researches on provenance, progeny and clone tests. Genetic diversity of each species can be saved by establishing collections (provenance trials, progeny tests, clonal archives, clonal seed orchard, seed bank, pollen and plant tissue banks) using this method.

In Croatia there are more than 350 forest seed objects: forest stands, seed stands, clonal seed orchard and group of tree. Croatian Forest Research Institute (CFRI), as official body, according Forest reproductive material legislative, supervises the production and marketing of forest reproductive material. Also Croatian forest research institute set up a register of forest seed objects constituting a gene bank of forest trees of Republic Croatia. Total area of all seed stands (category of seed: selected) in Croatia is 3'898.35 ha and for conservation of genetic diversity (gene bank) are suggest 1'103.60 ha (*in situ*). During the past 50 years, researchers Croatian Forest Research Institute were founded by dozens of provenance experiments. In this paper, we mention the provenance experiments involved in the gene bank of forest trees Croatia, where it is still carried out by scientific research like: Pedunculate oak provenance trials established in 1988, 2008 and 2010 (Gračan 1999, Perić et al. 2006, Ivanković et al. 2011), Silver fir provenance trials established in 2000 (Ivanković 2003) and International beech provenance trials established in 1998 and 2007 (Gračan and Ivanković 2001, Gračan et al. 2006, Jazbec et al. 2007, Ivanković et al. 2008).

In Table 5 is list of Clonal seed orchard nominated for registration in Genetic bank, while in Table 6 is list of provenance trials which are included in genetic bank (*ex situ*).

Table 5 - List of Clonal seed orchard nominated for registration in Genetic bank.

Species	No. of orchards	Type	Year of rising	Area (ha)
Pinus sylvestris	2	clonal	1966	3.0
Pinus nigra	2	clonal	2006	1.5
Larix europea	2	clonal	1985	2.5
Quercus robur	4	clonal	1996, 2000, 2001, 2008	47.0
Quercus petrea	1	clonal	2008	7.3
Alnus glutinosa	2	clonal	1985	1.7
Fraxinus angustifolia	2	clonal	2005, 2007	3.5
Prunus avium	1	clonal	2001	3.0
Pinus strobus	1	clonal	1965	-

According the same legislative, Croatian forest research institute take care about forming and conservation of reserve forest seed material in seed bank.

Table 6 - List of provenance trials which are included in Genetic bank.

Tree species	No. of trials	Year of establishing
Quercus robur	4	1988, 2008, 2010, 2010
Fagus sylvatica	2	1998, 2007
Abies alba	1	2000
Larix decidua	1	1959
Pinus sylvestris	1	1959
Pinus nigra	1	1959
Picea omorika	1	1959
Picea abies	1	1959

Conservation of forest trees genes represents maintenance of the evolutionary created adaptation potential of a particular species, i.e. its forest community and the entire forest ecosystem. For the purpose of conservation of forest genetic reso¬urces, we must protect the existing genetic variability, its adaptability to processes of natural evolution and forest tree breeding, as well as improve our knowledge and ways of identification of those individuals that have developed tolerance to certain diseases and pests. That way, we will be able to prevent a decrease in genetic resources of the endangered species. The research should be supplemented with data including making of species inventories, legislation, practical use, coordination on national and paneuropean level, together with promotion of public awareness on the importance of conservation of the endangered species in forest ecosystems.

Conclusions

Beside gene pool conservation and testing *in situ*- in natural populations in different sites, conditions for biodiversity testing as well as the familiarity with the range of potential variability *ex situ* are provided by establishing of separate plantations. Starting from the floristic, genetic and applicative potential of Serbian spruce, Norway spruce, Austrian pine, Balkan maple, Pedunculate Oak, European beech, the paper presents multi-annual researches aimed at becoming familiar with their gene pool as well as its conservation and directed utilization by the establishment of specialized plantations.

The obtained results enable better familiarity with the potential of production and adaptability of the analyzed species. Seed orchards and pilot seed forests, as specific plantations, should contribute to the conversion of potential genetic variability into the free one, as the base for directed utilization of tree gene pool, and serve as polygons for testing and preservation of biodiversity of these species.

The presented research objectives and methods and the results of the genetic valuation of forest tree species are up-to-date methods in gene pool conservation and testing, as well as planning and establishment of future plantation communities of these species.

The activities on conservation and use forest genetic resources lead to produce superior genotypes which are important for increasing of wood production and climate change mitigation. Those activities support global bio-economy by enhancement of use genetic potential of forest trees.

References

Andonoski A. 1988 - *The state of Macedonian forest genofond and measures for its conservation*. In: X Congress of dendrologists, Sofia, Bulgaria: 144-152.

Andonoski A. 1974 - *Application of genetical principles on the production of forest seeds*. Forest review 5,6: 43-67, Skopje, Macedonia.

Andonoski A. 1994 - *Genetics and tree improvement*. Skopje, Macedonia: 226-261.

Andonoski A. 1995 - *Adaptive capability of some introduced forest tree species*. Master thesis: 82-94, Skopje, Macedonia.

Andonoski A. 1998 - *Multy-Country Forestry Program (National report for the Republic of Macedonia)*, Skopje, Macedonia:18-34.

Andonovski V., Velkovski N. 2011 – *Conservation of Macedonian pine (Pinus peuce Griseb.) genetic resources in Pelister National Par.* Journal of Protected Mountain Areas Research and Management 3 (1): 49-53.

Ćurčić G., Isajev V., Tošić M. 1999 - *Generativna semenska plantaža planinskog javora (Acer heldreichii Orph.) kod Ivanjice – pilot objekt – za dalje oplemenjivanje vrste*. Drugi Kongres Genetičara Srbije. Knjiga Abstrakta 215.

Ćurčić, G. 1997- *Generativna semenska plantaža planinskog javora (Acer heldreichii Orph.) "Perkovići-Strane" kod Ivanjice*. Diplomski rad. str. 1-43. Beograd.

Erdeši J. 1996 - *Vegetativna semenska plantaža hrasta lužnjaka (Quercus robur L.) – izvođački projekat*. JP " Srbijašume„ Beograd, Šumsko gazdinstvo Sremska Mitrovica. Sremska Mitrovica.

European Commission's Seventh Framework Programme (FP7) 2008 – *The European Bioeconomy in 2030, BECOTEPS –Bio-Economy Technology Platforms:* 5-22.

Food and Agriculture Organization of the United Nations 2014 - *The State of the Worlds Forest Genetic Resources*. FAO Rome.

Galović V., Orlović S., Zorić M., Kovačević B., Vasić S. 2014 - *Different phenology induced genotype diversity of Q. robur L. in the seed orchard in Srem provenance, Republic of Serbia*. International Conference "Natural reseources, green technology, and sustainable development „, Zagreb, November 26-28, 2014 (in print).

Gračan J., Ivanković, M. 2001 - *Prvi rezultati uspijevanja provenijencija obične bukve (Fagus sylvatica L.)* u Hrvatskoj. Znanost o potrajnom gospodarenju Hrvatskim šumama / Matić, Slavko; Krpan P. B. Ante; Gračan Joso (ur.). Zagreb: Šumarski fakultet; Jastrebarsko, Šumarski institute: 636.

Gračan J., Ivanković M., Marjanović H., Perić S. 2006 - *Istraživanje uspijevanja provenijencija domaćih i stranih vrsta drveća s osvrtom na međunarodni pokus provenijencija obične bukve (Fagus sylvatica L.)*. Radovi. Izvanredno izdanje 9: 337-352.

Gustafsson A. 1950 - *Conifer Seed Plantations: Their Structure and Genetical Principles*. Proceedings of the III World Forestry Conres No 3, Helsinki: 128-138.

Isajev V. 1987 - *Oplemenjivanje omorike (Picea omorika /Panč./ Purkyne) na genetičko selekcionim osnovama*. Doktorska disertacije. Šumarski fakultet Beograd: 285-381.

Isajev V., Delić S., Mančić A., Šijačić M. 1995 - *Unapređenje osnovnih Šumskih kultura u funkciji razvoja Srbije*. Potencijali šuma i šumskih područja i njihov značaj za razvoj Srbije. Šumarski fakultet Beograd: 63-69.

Isajev V., Đukić M. 1990 - *Značaj provenijeničnih testova za vrednovanje genetsko-fiziološkog kvaliteta sastojina smrče na Kopaoniku*. Zbornik radova sa nučno-stručnog skupa "Priroda Kopaonika- zaštita i korišćenje: 223-235.

Isajev V., Šijačić M., Vilotić D. 1994 - *Varijabilnost makroskopskih i mikroskopskih karakteristika dvogodišnjih sadnica 26 familija polusrodnika planinskog javora (Acer heldreichii Orph.)*. Šumarstvo 3-4: 21-28.

Isajev V., Tucović A. 1992 - *Provenijenični test smrče na tri lokaliteta kod Ivanjice*. Izvođački projekat Beograd: 1-52.

Isajev V., Tucović A., Šijačić-Nikolić M. 1998 - *Očuvanje i unapređenje korišćenja genofonda endemoreliktnih vrsta četinara Srbije*. Zaštita prirode 50: 327-334.

Ivanković M. 2003 - *Varijabilnost nekih svojstava obične jele (Abies alba Mill.) u pokusu provenijencija "Brloško"*. Radovi Šumarski institut, Jastrebarsko, 38: 159-176.

Ivanković M., Bogdan S., Božič G. 2008 - *Varijabilnost visinskog rasta obične bukve (Fagus sylvatica L.) u testovima provenijencija u Hrvatskoj i Sloveniji*. Šumarski list 11-12: 529-541.

Ivanković M., Popović M., Bogdan S. 2011 - *Varijabilnost morfoloških svojstava žireva i visina sadnica hrasta lužnjaka (Quercus robur L.) iz sjemenskih sastojina u Hrvatskoj*. Šumarski list: 46-58.

Ivetić V. 2004 - *Uticaj staništa i provenijencija na razvoj juvenilnih kultura smrče (Picea abies /L./ Karst) na Goliji*. Magistarski rad. Šumarski fakultet: 1-131.

Jazbec A., Šegotić K., Ivanković M., Marjanović H., Perić S. 2007 - *Ranking of European beech provenances in Croatia using statistical analysis and analytical hierarchy process*. Forestry 80(2): 151-162.

Jovanović M. 1972 - *Proizvodnja šumskog selekcionisanog semena u semenskim plantažama*. Aktuelni problemi šumarstva, drvne industrije i hortikulture: 191-199.

Kajba, D., Gračan, J., Ivanković M., Bogdan S., Gradečki-Poštenjak M., Littvay T., Katičić I. 2006 - *Očuvanje genofonda šumskih vrsta drveća u Hrvatskoj*. Glasnik za šumske pokuse. 5; 235-249.

Kleinschmit J. 1970 - *Present knowledge in spruce provenance and species hybridiation potential*. IUFRO Norway spruce meeting, Bucharest, S 2.03.11- S 2.02.11: 187-201.

König A.O. 2005 - *Provenence research:evaluating the spatial pattern of genetic variation*. In: Conservation and Management of Forest Genetics Resources in Europe. Arbora Publishers, Zvolen: 275-290.

Lines R. 1967 - *Standardiyation of methods for provenence research and testing*. Proceedings 14th IUFRO Congr. 3: 672-718.

Mataruga M. 1997 - *Međuzavisnost osobina i razvoja sadnica crnog bora (Pinus nigra Arn.) u semenskoj plantaži na Jelovoj Gori*. Magistarski rad: 1-112.

Mataruga M., Isajev V. 1998 - *Mogućnost testiranja i očuvanja biodiverziteta crnog bora u specijalizovanim kulturama*. Zaštita prirode 50: 63-71

Nikolić N., Krstić B., Pajević S., Orlović S. 2006 - *Varijabilnost osobina lista kod različitih genotipova hrasta lužnjaka (Quercus robur L.)*. Zbornik Matice srpske za prirodne nauke 111: 95-105.

Nikolić N., Merkulov Lj., Krstić B., Orlović S. 2003 - *A comparative analysis of stomata and leaf trichome characteristics in Quercus robur L. genotypes*. Zbornik matice srpske za prirodne nauke 105: 51-59.

Nikolić N., Merkulov Lj., Pajević S., Krstić B. 2005 - *Variability of leaf anatomical characteristics in Pedunculate oak genotypes (Quercus robur L.)*. Gruev, B., Nikolova, M., Donev A. 2005 - Proceedings of the Balkan scientific conference of biology, Plovdiv, Bulgaria, 19-21, May 2005: 240–247.

Nikolić N.P., Merkulov L.S., Krstić B.Đ., Pajević S.P., Borišev M.K., Orlović S. 2010 - *Varijabilnost anatomskih osobina žira kod genotipova hrasta lužnjaka (Quercus robur L.)*. Zbornik Matice srpske za prirodne nauke 118: 47-58.

Nikolić N.P., Orlović S. 2002 - *Genotipska varijabilnost morfoloških osobina žira hrasta lužnjaka (Quercus robur L.)*. Zbornik Matice srpske za prirodne nauke 102: 53-58.

Orlović S., Radivojević S., Erdeši J., Obućina Z., Janjatović, G. 1999 - *Seed orchards of intra-species forms as a way for maintain and increasing genetic variability of Pedunculate oak in Yugoslavia*. International Conference "Recent advances on oak health in Europe", Warsaw, Book of abstracts 31.

Orlović S., Erdeši J., Radivojević S., Janjatović G. 2001 - *Semenske plantaže hrasta lužnjaka (Quercus robur L.) – osnov za dalje oplemenjivanje u ravnom Sremu*. Šumarstvo 1: 1-9.

Orlović S., Klašnja B., Galić Z., Pilipović A. 2002 - *Conservation of Pedunculate oak (Quercus robur L.) in Yugoslavia*. Proceedings DYGEN Conference «Dynamics and conservation of genetic diversity in forest ecosystems»: 210.

Perić S., Jazbec A., Medak J., Topić V., Ivanković M. 2006 - *Analysis of biomass of 16th Pedunculate Oak Provenances*. Periodicum Biologorum Vol. 108, No 6, 631-709.

Rajora, O.P., Mosseler A. 2001. - *Challenges and opportunities for conservation of forest genetic resources*. Euphytica 118 (2): 197-212.

Skrøppa T. 2005 - *Ex situ conservatin methods*. In: Conservation and Management of Forest Genetics Resources in Europe. Arbora Publishers, Zvolen: 567-583.

Stojnić S., Orlović S., Pilipović A., Kebert M., Šijačić-Nikolić M., Vilotić D. 2010 - *Variability of physiological parameters of different European beech provenances in international provenance trials in Serbia*. Acta Silvatica & Lignaria Hungarica 6: 135-142.

Stojnić S., Orlović S., Galić Z., Vasić V., Vilotić D., Knežević M., Šijačić-Nikolić M. 2012a - *Stanišne i klimatske karakteristike u provenijeničnim testovima bukve na Fruškoj gori i u Debelom Lugu*. Topola 189/190: 145-162.

Stojnić S., Orlović S., Pilipović A., Vilotić D., Šijačić-Nikolić M., Miljković D. 2012b - *Variation in leaf physiology among three provenances of European beech (Fagus sylvatica L.) in provenance trial in Serbia*. Genetika 44: 341-353.

Stojnić S. 2013a - *Varijabilnost anatomskih, fizioloških i morfoloških karakteristika različitih provenijencija bukve u Srbiji*. Doktorska disertacija, Univerzitet u Beogradu, Šumarski fakultet: 340p..

Stojnić S., Sass-Klaassen U., Orlović S., Matović B., Eilmann B. 2013b - *Plastic growth response of European beech provenances to dry site conditions*. IAWA Journal 34: 475-484.

Šijačić-Nikolić M., Milovanović J. 2010 - *Konzervacija i usmereno korišćenje šumskih genetičkih resursa*. Univerzitet u Beogradu Šumarski fakultet, Beograd, 200p..

Štajner D., Orlović S., Popović B., Kebert M., Stojnić S., Klašnja B. 2013 - *Chemical parameters of oxidative stress adaptability in beech*. Journal of Chemistry, Article ID 592695, doi:10.1155/2013/592695.

Tucović A., Isajev V. 1982 - *Uticaj različitih tipova oprašivanja na neka svojstva šišarica i semena omorike*. Glasnik Šumarskog fakulteta, Serija C Pejzažna arhitektura 59: 59-65.

Tucović A., Isajev V. 1983 - *Neka uporedna opažanja u kulturama omorike odgajenim na različitim staništima SR Srbije*. Glasnik Šumarskog fakulteta, Serija A. 60: 77-87.

Tucović A., Isajev V. 1991 - *Metapopulaciona strategija osnivanja generativnih semenskih plantaža drveća*. Zbornik radova Prošlost, sadašnjost i budućnost srpskog šumarstva kao činioca razvoja Srbije: 313-323.

Von Wuehlisch G., Ballian D., Bogdan S., Forstreuter M., Giannini R., Götz B., Ivankovic M., Orlovic S., Pilipovic A., Sijacic Nikolic M. 2010 - *Early results from provenance trials with European beech established 2007*. COST E52 "Evaluation of Beech Genetic Resources for Sustainable Forestry" Final Meeting. Book of abstracts. 4-6th May 2010, Burgos, Spain: 21.

Von Wuehlisch, G. 2004 - *Series of International Provenance Trials of European Beech*. Proceedings from the 7th International Beech Symposium IUFRO Research Group 1.10.00 "Improvement and Silviculture of Beech". 10-20 May 2004, Tehran, Iran: 135-144.