



Variability and breeding of beech (*Fagus moesiaca/Domin, Maly/Czeczott.*) in Serbia

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Abstract

Beech breeding is mostly directed to the conservation of genetic resources, the selection of the corresponding provenances for different sites, enhanced production and trade of planting stock, study of the species evolution development, and the establishment of live archives and seed orchards. The biochemical and molecular analyses based on the selected population and individuals of different beech stands in Serbia concluded that the adaptation and survival at population level are favored by higher genetic diversity whereas at the individual level it is favored by higher degree of heterozygosis. Breeding of beech in Serbia, depending on the current requirements of forestry and on the state of forests, should be performed in two directions: in the direction of improving the natural populations and in the direction of obtaining the new selected beech genotypes and hybrids, which will have superior target characteristics than the existing types. The previous research shows that there are genetic differences between beech populations in Europe and Serbia. This requires the programs of closer research and of directed application of beech genetic variability.

Key words: beech, variability, breeding, Serbia

1. Introduction

The economic significance, as well as the general biological-ecological characteristics of beech (Ertekin et. al., 2015) has made this species in its range the subject of interest of a great number of scientists and professionals for more than a hundred years. Based on the study results of bioecological, production and technical-physics characteristics of beech wood, it was decided to investigate the cause of the recorded ecological, morphological and physiological variability also by the methods of genetics and plant breeding (Isajev, 2005). The choice of basic methods and the corresponding variants of the basic methods of beech breeding in Serbia depended on: 1. the study of its bioecological characteristics; 2. coeno-ecological characteristics of its populations; 3. assignments that are to be worked out; 4. importance of their solution for economy.

The general ecological features of the region in Serbia where pure and (or) mixed beech populations occur spontaneously in interaction with their gene pool, condition the occurrence of the expressed variability of a major number of its morphological and genetic-physiological characteristics. As Moesian beech - (*Fagus moesiaca/Domin, Maly/* is distinguished by high individual and group variability, and as it has numerous intraspecific taxa – varieties, biotypes and ecotypes, this requires a multidisciplinary and complex approach in further work on the research and the directed use of group and individual variability.

This paper surveys the results of the study of genetic potential and variability of beech in Serbia. The presented results are based on longterm analyses of individual and group variability of beech morphological and physiological characters; comparative studies by provenance tests; the review of experiences in the implementation of the results of biochemical and molecular analyses at the level of the selected populations and individuals of different provenances.

2. Materials and methods

The protection and directed use of the most valuable beech genetic resources is realised mostly:
in situ - by the selection of the best beech natural populations and by the revision of the existing and the selection of the new seed stands, groups, individual trees, and
ex situ – by the establishment of provenance tests, live archives, clonal and seedling seed orchards.

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By the implementation of the method of simple mass selection, 19 beech seed stands were designated with the total area of 137.57 ha. Parallel with the mass selection, individual selection was also undertaken, the selection of test trees for the initial material in the application of synthetic methods of breeding. In this aim, on 17 sites in the central region of Serbia, 74 test trees were selected, of which one was in the sub-alliance *Fagenion moesiaca submontanum* Jov., 1976, 40 individuals were in the sub-alliance *Fagenion moesiaca montanum* Jovanović M. 1976, and 33 trees were in the sub-alliance *Abieti-Fagenion moesiaca* Jovanović M., 1976. The test trees were merely evaluated based on their phenotypes. The more precise insight into their genotypes was to be attained in the later phase of research by early tests on the generative progeny. In this way it will be checked if really the best specimens of this species in Serbia have been selected. In the aim of their conservation, it was decided to protect them from felling and to propagate them asexually in a sufficient number so that the produced vegetative copies, which contain the same genetic base as parent trees from which they originate, could be used in further breeding. Therefore, four methods of grafting were researched comparatively, Jovanović M. 1971:

- a) method of side grafting on overtopped rootstocks, using normal buds;
- b) method of side grafting on overtopped rootstocks, using dormant buds;
- c) method of side grafting on non-overtopped rootstocks using normal buds and
- d) method of wedge grafting, using normal buds (Figure 2).

In auto-vegetative propagation of beech with air layering (Figure 8), using growth stimulators such as beta-indole acetic acid at therates of 0.5 and 1.0% and beta-indole butyric acid at 0.5%;

Genetic variability of common beech was studied using genetic markers in six autochthonous populations, aging from 110 to 160 years which represented the cross section of the stands at the altitude (550-960 m). The samples for these analyses were the protein markers from beechnut, and the applied method was electrophoresis, which is a simple and fast analytic method. The genetic component of the research is based on the analysis of RAPD markers from bulk samples, using 28 primers. The spatial component of the research is based on the geographical position of the studied populations. Grouping of the studied populations in the regions, as well as their separation, was performed using the Monmonier's algorithm of maximum differences. To visualize the results and mapping the regions of beech provenances in Serbia, GIS was used, with database included the results of this study.

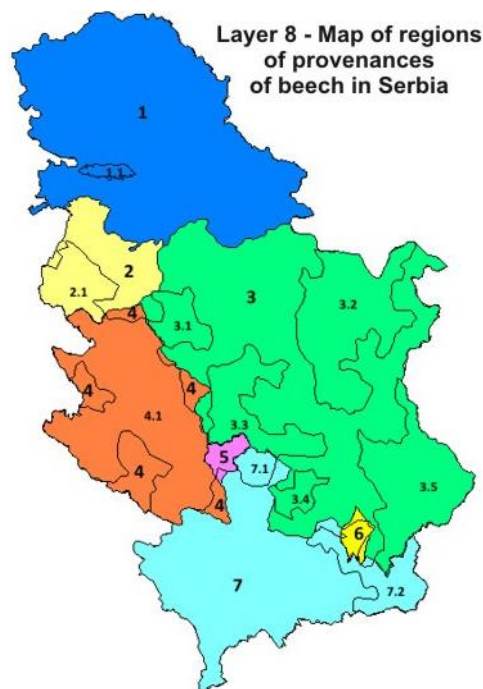
3. Results

Group variability of beech in Serbia was studied in detail by mass selection. Seed stands were designated and the quality of phenotype characteristics of trees was evaluated and studied (Glišić, 1960; Jovanović, 1961; 1971; Marić *et al.*, 1961, 1962; Tucović, 1970, 1976; Isajev *et al.*, 2003). These papers improved the basic guidelines for the evaluation, selection and management of seed stands. The number of selected beech seed stands depended on the phenotypic characters of the trees and the ecological diversity of the sites of beech forests. The species with an extensive range of distribution, such as beech, have a greater genetic differentiation of the population which spontaneously occur at different localities. When the species is characterised by a marked variability of continuous and discontinuous characters, it is necessary to select a greater number of seed stands. Based on the method of simple mass selection, 19 beech seed stands were selected, total area 137.57 ha, Figure 1.



Figure 1. Seed stand of beech in MU Kukavica, compartment 54 Vladičin Han (By photo V. Ivetić, 2001).

The spatial pattern of the seed stand localities reflects almost completely the coeno-ecological, ecological and population diversity of beech in Serbia. The results of spatial analysis of genetic diversity have practical application in the definition and delineation of regional provenances of forest trees (Ivetić et al. 2008; 2010; 2012). Research was based on the material from 27 natural populations of beech in Serbia. The genetic component of the research is based on the analysis of RAPD markers from bulk samples, using 28 primers. The spatial component of the research is based on the geographical position of the studied populations. Grouping of the studied populations in the regions, as well as their separation, was performed using the Monmonier's algorithm of maximum differences, Monmonier, M.S., 1973. To visualize the results and mapping the regions of beech provenances in Serbia, GIS was used, with database included the results of this study. Combining of GIS tools with molecular genetics technology increases the strength of results, by using the spatial dimension of information they provide and thus provides an alternative perspective that may lead to a better understanding of genomic functions. Visualization (spatial analysis research) and presentation (mapping) the spatial distribution of genetic data is likely to emphasize the patterns of diversity and thus further improve the interpretation of results. This map was used to compare genetic to geographic barriers, as well as drawing maps of regions of provenances map 1. Furthermore, spatial analysis may allow the detection of relations between the regions of the genome, and habitat characteristics which surrounding the populations.



Map 1. Delineation of regions of beech provenances in Serbia, based on Monmonier's geographical barriers obtained from corrected genetic distance (Ivetić et al., 2012).

Based on the above, it can be expected that the collected seeds and the produced planting material will encompass the genetic potential of beech in Serbia. Our previous experience, gained by the beechnut collection and trade, indicates that the best results are achieved when its collection, processing and trade are organised at the local level.

In each beech seed stand the following is organised and executed:

- 1) short-term and
- 2) long-term activities, which include also the planning of the adequate structure of personnel, equipment and material.

The isoenzymatic analysis resulted in the information on the genetic base of the adaptation of common beech populations to the sites at different altitudes. There are different genetic structures among the populations, and the frequency of individual alleles changes significantly from the lower towards the higher altitudes. The results of the above studies indicate that the reproductive material from beech stands from the lower altitudes, before the transfer to the higher altitudes, should be controlled by tests and vice versa. The above genetic specificities of beech populations should be taken into account in the activities directed to the conservation beech genetic resources.

The study results support the hypothesis that beech during the ice age was present in the micro-refuges in the south-east Alps and in a part of Slovenia. The populations from Central and Southeast Europe can be divided into two different groups: the first and greater group consists of the populations from the northwest part of the study area, while the second group consists of the populations from the east Balkan Peninsula. The differentiation in that part could confirm the existence of a special taxon on Balkan, the subspecies *Fagus sylvatica subsp. moesiaca* (Maly) Czecht.,

Gömöry *et al.*, 1999. Based on the results of biochemical and molecular analyses at the level of the selected populations and individuals of different beech provenances in Europe, it can be concluded that, in the changed ecological conditions, the ability of adaptation and survival at the level of populations, was favoured by a higher genetic variability, and at the individual level, by a higher degree of heterozygosis.

The analysis of genetic potential of test trees also used the methods of heterovegetative and auto-vegetative propagation. The most frequently applied method was the heterovegetative propagation – grafting, mostly performed in the glasshouses of the Institute of Forestry and the Faculty of Forestry in Belgrade.

In grafting, special attention was focused on the following properties: quality of rootstock and scions, time of taking, length and method of keeping the scions till the moment of grafting, age of wood for scions, as well as the grafting method.

The grafting (Figure 2) in the glasshouses started in the middle of March and in the open in the first half of April. It ended by the end of April, more rarely at the beginning of May. During the summer grafting in the open, when the budding method was applied, the optimal time for grafting was the beginning or the middle of the second half of August, and it lasted for 7-10 days.



Figure 2. One- year- old grafts of beech produced by triangulation (By photo: M. Jovanović)

In auto-vegetative propagation of beech by air layers, Figure 3, using growth stimulators such as beta-indole acetic acid in concentrations 0.5 and 1.0% and beta-indole butyric acid in concentrations 0.5; 1.0 and 2.0%, the results were excellent, i.e. the percentage of rooting was from 90 to 100%,(Jovanović, 1971). Somewhat poorer results - success 65-85% - were achieved by alpha-naphthyl acetic acid in concentrations 0.1 and 0.2%. The test of the significance of differences shows a significant difference at the level of 1.0% between 0.5% concentration of beta-indole acetic acid, in which rooting was 100%, and 0.1% concentration of alpha-naphthyl acetic acid, in which the percentage of rooting accounted for 65%.



Figure 3. Developed root system on an air layer (By photo M. Jovanović)

4. Conclusions and discussion

The aims of the analysis of group and individual variability of beech in Serbia were the following: to understand and conserve the genetic resources of this species, to select the adequate provenances at different sites, to enhance the production and trade of seed and planting material, to explain the evolution development of the species, and to establish the live archives and seed orchards.

Grouping of the studied populations in the regions, as well as their separation, was performed using the Monmonier's algorithm of maximum differences. To visualize the results and mapping the regions of beech provenances in Serbia, GIS was used, with database included the results of this study.

Based on the study results of the biochemical and molecular analyses at the level of the selected populations and individuals of different beech provenances, it can be concluded that the adaptation ability and survival ability at the level of populations was favoured by the higher genetic variability, and at the individual level – by the higher degree of heterozygosis.

In hetero-vegetative propagation of beech test trees, the best results were achieved by the method of side grafting on overtopped, non-overtopped rootstocks, and by the method of wedge grafting, using normal buds.

In auto-vegetative propagation of beech by air layering, using growth stimulators, such as beta-indole acetic acid in concentrations 0.5 and 1.0% and beta-indole butyric acid in concentrations 0.5; 1.0 and 2.0%, the results were very good, i.e. the percentage of rooting was from 90 to 100%.

The information on the genetic variability of beech is important for the more precise study of:

- intra-population and inter-population differentiation in its range of distribution,
- adaptation ability, and
- existence and the degree of divergence of individual beech provenances at different sites.

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