Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 6 (55) No. 1 - 2013

GENETIC DIVERSITY OF ROMANIAN POPULATIONS OF *FAGUS SYLVATICA* – A REVIEW

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Abstract: *Genetic diversity is very important to species evolution in changing environments. The aim of this study is to review the work done on genetic diversity of Romanian populations of beech, estimated mainly with genetic markers. Only a few studies have been conducted so far on Romanian beech populations. A large scale cpDNA study revealed that the populations of beech from the Romanian Carpathians have their origin in Moravian population. Several isolated populations have not been analyzed so far and could bring new data about the evolutionary history of this species. Due to the increase of temperature in the south-eastern Romania marginal populations of beech are currently growing under extreme conditions. A comparative study between core and marginal populations could bring new insights into the adaptation of beech to dry conditions.*

Key words: *beech, isozyme, microsatellite, genetic diversity, cpDNA.*

1. Introduction

The common beech (*Fagus sylvatica* L.) is one of the most important forest trees in Europe. It occurs in Central, Southern and Western Europe, being a plain, hilly or mountain species across its natural distribution range [2]. In Romania beech forests cover about 2 million hectares and have high ecological and economic value [22].

Genetic variation in beech populations was studied in provenance trials and by means of genetic markers: isozyme and molecular markers (AFLP, RFLP, RAPD, SSR, SNP, EST) [31]. The conservation of the genetic diversity is very important for the survival of species and for adaptation to climate change conditions [1], [18].

There have been only a few analyses regarding the genetic diversity of beech in Romania. The aim of this study is to review the studies on genetic diversity in beech populations with focus on the Romanian populations.

2. Isoenzyme variability

Isoenzyme markers are electrophoretically detectable forms of enzymatic proteins visualized by substrate-specific staining and there are alternative enzyme forms encoded by different alleles at the same locus, which can be used as genetic markers [21].

The first studies [9] assessing the genetic diversity of beech began later than for the conifers, in 1979 there being identified two

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polymorphic loci, LAP-A and ACP-A, with four alleles each. In 1982, three enzymatic genetic markers, two in peroxidases (PER) and one in glutamate oxaloacetate transaminases (GOT), were described [26].

Most studies regarding the beech genetic variability estimated by isoenzymes are based on the methodology proposed by Müller-Starck and Starke in 1993 [15] who suggested the nomenclature for 20 loci – *Aco-A*, *Aco-B*, *Dia-A*, *Gdh-A*, *Got-A*, *Got-B*, *Idh-A*, *Lap-A*, *Lap-B*, *Mdh-B*, *Mdh-C*, *Ndh-A*, *Per-B*, *Pepca-A*, *6Pgdh-A*, *6Pgdh-B*, *6Pgdh-C*, *Pgi-B*, *Pgm-A*, *Skdh-A*, including 78 codominant alleles. The enzymes were extracted from embryos, buds, young leaves or pollen and separated from crude homogenate by horizontal starch gel electrophoresis. Another study about genetic differentiation of beech populations in Eastern Europe was based on 12 isozyme loci, locus Mdh-1 was monomorphic in most of the populations and the overall mean number of alleles was 2,21, with slight differentiations compared to the value obtained in the southeastern Europe [17]. In 1995, Konnert analysed the genetic variation of beech in Bavaria with 16 isoenzyme gene loci and concluded that the variability was higher at the intra-populational level compared to the inter-population one [10].

Leonardi and Menozzi [12] investigated the genetic structure for 21 Italian populations and the role of postglacial recolonization. They found a higher level of genetic variability in southern Italy, which denotes the southern origin of beech and outlines the northward recolonization of this. The hypothesis was to consider a correlation between allele frequencies and altitude. In reality, in most of the populations the allele frequencies either did not vary with altitude or the correlation was weak [4].

By using isoenzymes analysis and by correlating the resulted genetic data with the empty seeds percentage, the main cause of the appearance of beech empty seeds was established, being the presence of self-fertilization [28].

A recent study [23] to assess genetic diversity of European beech in Poland showed that the genetic differentiation was comparable to another one obtained in another region of Europe. The values of N_a – the number of alleles per locus, H_o – observed heterozygosity, H_e – expected heterozygosity, listed in Table 1, were highly similar to those obtained in Romania.

Table 1

Summary of genetic variation [23]

Nr.	Region	N_a	H_o	H_e
1	West Slovakia	2.8	0.269	0.280
2	East Slovakia	2.8	0.258	0.263
3	**Romania**	**3.3**	**0.246**	**0.263**
4	Ukraine	2.4	0.251	0.285
5	Czech Moravia	2.5	0.254	0.271
6	Poland	3.1	0.246	0.262

3. The chloroplast genetic diversity

In angiosperms, the chloroplast DNA is maternally inherited. The uniparental mode of transmission allows estimation of gene flow or seed migration in the species evolution [27].

The first studies on the genetic diversity of beech assessed using chloroplast DNA (cpDNA) markers were made in 1996 [5].

There were analyzed 85 populations from Europe and were identified 11 haplotypes, the haplotype 5 being the most frequent. In the other regions of Europe there were observed a few rare haplotypes, haplotype 9 in Spain, haplotype 8 in Hungary and haplotypes 4 and 7 in the Pyrenees.

However, the genetic data indicate the existence of two refugia during the last glaciation, a refugium in the Balkan peninsula (where there are present haplotypes 10 and 11) and another one in the south of the Italian peninsula (haplotypes 1, 2 and 3) [5], [24].

The geographical structure of genetic variation at chloroplast DNA was investigated in Europe with chloroplast DNA markers. 352 populations were analyzed with RFLP markers. Two chloroplast DNA fragments, trnD - trnT (DT) and ORF184 - petA (OA), each restricted to a specific enzyme were analyzed. In the same study, 468 populations were investigated with six microsatellite chloroplast markers, namely *ccmp2*, *ccmp3*, *ccmp4*, *ccmp6*, *ccmp7* and *ccmp10*. They identified 20 haplotypes using PCR – RFLP and 24 haplotypes using SSRs markers [14].

The distribution of PCR – RFLP haplotypes indicates the presence of haplotypes 1 and 2 in Europe, haplotypes 3 and 4 in Italian Peninsula. In the southern part of the Balkan Peninsula Magri *et al.* identified the haplotypes 17-20, the high number haplotypes reflect the presence of *Fagus orientalis* and/or its hybridization with *Fagus sylvatica*.

By analyzing the distribution of chloroplast microsatellite haplotypes there has been observed the existence of the haplotype 2 in Italy, a high number haplotypes (haplotypes 7-20) in Balkan Peninsula and one haplotype (haplotype 1) on the other European regions. In Romania, the PCR-RFLP haplotypes 1 and 2 and the chloroplast microsatellite haplotype 1 are present.

After the interpretation and the correlation of the palaeobotanical and genetic data a possible location of refugium for *Fagus sylvatica* and the colonization routes during the post-glacial period were found. Possible refugia could be in Iberian Peninsula, France, Italian Peninsula, Slovenia, Balkan Peninsula, Moravia and Romania (Apuseni Mountains).

4. Genetic diversity of beech populations assessed with nuclear microsatellite markers

Microsatellites or simple sequence repeats (SSR) are tandem repeats of 1-6 nucleotides found in the nuclear genomes [21]. The microsatellite markers were used to uncover the patterns of genetic diversity, to estimate the effects of pollen and seed flow, the species ecological adaptive processes and for the seed source identification [7], [25].

EST-SSRs (Expressed Sequence Tag) are microsatellites located in coding regions. They are also available for many tree species and their advantages consist in their higher transferability between species [19].

In 2003, seven primers for the amplification of microsatellite markers in *Fagus sylvatica* and *Fagus orientalis* were developed, all showing a high level of polymorphism [16].

The spatial distribution of genetic variation in beech population was characterized with microsatellite markers, developed initially for *Fagus japonica* and *Fagus crenata*. Only for three microsatellite markers, *mfc5, mfc9-2, mfc11*, different numbers of alleles were observed for *Fagus sylvatica* [28].

Buiteveld and his colleagues [3] analyzed the genetic diversity and structure of European beech with four microsatellite markers in ten beech stands located in Europe. They wanted to observe the impact of forest management on genetic diversity. Because there were no significant differences in effective number of alleles, heterozygosity and expected heterozygosity, they concluded that the forest management does not influence the genetic diversity [3].

Another study carried out in Germany [18] used microsatellite and AFLP markers to determine the impact of forest management activities on spatial genetic structure of beech in 29 stands (10 unmanaged stands and 19 managed stands). For this, they used six SSRs markers originally developed for *F. sylvatica* and three EST-SSRs originally developed for *Quercus* spp. in three multiplexing sets and concluded that forest management activities reduced the intensity of fine-scale genetic structure in the beech stands.

Recently, two multiplex sets of microsatellite (SSR) markers allowing rapid amplification of 16 polymorphic loci have already been developed for *Fagus sylvatica* [11].

5. Conclusions

Isoenzymes and molecular markers were used to establish the migration routes, to estimate the relatedness of individuals, or to determine the spatial genetic structure and the impact of forest management. Romanian marginal populations of beech may contain original genetic combinations of high value to adapt beech forests to climate change. In this context it would be interesting to compare the nuclear genetic structure of beech populations situated at the hot margins of its distribution with core beech populations. Another direction of investigation would be to verify the hypothesis of existence of a small glacial refugium is Apuseni Mountains.

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