Growth Tendency of *Quercus robur* L. Provenances in Bosnia and Herzegovina Provenance Test with Relation to Fixation Index

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Abstract

**Aim of study:** Specific genetic structure of the remaining populations of pedunculate oak (*Quercus robur* L.) in Bosnia and Herzegovina makes them significant for the preservation of the diversity of this species in Bosnia and Herzegovina and Europe. For this reason, we established provenance test in 2009. The aim of the study is to determine the correlation between provenance growth and fixation index. The ultimate goal is the reintroduction of the species in suitable areas.

**Material and method:** The research included measuring the height and the root collar diameter of four-, six- and eight-year-old plants in the provenance test and molecular analyses using isoenzymes.

**Main results:** Variance analysis showed statistically significant differences between populations in all tested characteristics, which was confirmed by the Duncan test. Provenances of Miljevina Foča, Stojčevac, and Visoko Muhašinovići are at the bottom of the list with the lowest growth of four-, six-, and eight-year-old plants. Provenances of Drvar, Mutnica Cazin, Kaćuni, and Jelah showed the best growth.

**Highlights:** Eight provenances showed positive fixation index values, particularly Miljevina Foča, Bosanska Dubica, and Drvar. Positive fixation index value of Miljevina provenance matches its low growth, while this is not the case in Drvar and Bosanska Dubica provenances.

**Keywords:** Pedunculate Oak, Provenance Experiment, Morphologic Variability, Genetic Variability, Fixation Index

Bosna Hersek Orijin Denemelerinin Fiksasyon İndeksiyle İlişkisine Göre *Quercus robur* L. Orijinin Büyüme Eğilimi

Öz

Çalışmanın amacı: Günümüzde Bosna-Hersek'te saplı meşe (*Quercus robur* L.) popülasyonlarının küçük ve dağınık olmasına rağmen, bu türün spesifik genetik yapısı, saplı meşe çeşitliliğinin korunması için önemlidir. 2009 yılında Bosna-Hersek Žepče'de saplı meşe orijin denemeleri kurulmuştur. Bu arastırmamızın amacı, orijinlerin büyümü ve fiksasyon indeksi arasındaki ilişkiye tespit etmek ve Bosna Hersek'teki diğer saplı meşe orijinlerinin kalitesi hakkında bilgi sahibi olmaktır.

**Materyal ve yöntem:** Orijin denemeleri süresince, 4, 6 ve 8 yaşındaki bitkilerin boyu ve kök boğazı çapı (2012, 2014 ve 2016 yılları bahar aylarında) ölçülmüştür. Ayrıca, 28 orijinin tamamında hayatta kalan bitkiler ölçülümlüdür.

**Sonuçlar:** Orijin denemelerindeki bütün bitkilerin ortalamada boyu 2012 yılında 50.3 cm, 2014 yılında 117.9 cm ve 2016 yılında 168.7 cm olduğu tespit edilmiştir. Dört yıllık bitkilerde yürütülen orijin denemelerinde tüm bitkilerin ortalamada kök boğazı çapı 13.1 mm, altı yıllık bitkilerde 28.9 mm ve sekiz yıllık bitkilerde 42.8 mm olarak belirlenmiştir. İstatistiksel analiz sonuçlarına göre popülasyonlar arasında anlamli farklılıklar tespit edilmiştir. Miljevina Foča, Stojčevac, ve Visoko Muhašinovići orijinlerinin 4, 6 ve 8 yıllık fidanları en düşük büyümeye sahiptir, Drvar, Mutnica Cazin, Kaćuni, ve Jelah orijinleri en iyi gelişmeyi göstermiştir.

**Onemli vurgular:** Yırlı sekiz orijinden 8’i, pozitif fiksasyon indeksi değerleri göstermiştir. Özellikle Miljevina Foča, Bosanska Dubica ve Drvar orijinleri vurgulaması gereken pozitif fiksasyon endeksi değerlerine sahiptir. Miljevina Foča orijini öne çıkarırken; fiksasyon indeksi zayıf büyüme ile eşleşırken, Drvar ve Bosanska Dubica durum farklıdır.

**Anahtar kelimeler:** Saplı Meşe, Orijin Denemesi, Morfolojik Değişkenlik, Genetik Değişkenlik, Fiksasyon İndeksi.
Introduction

The area of Bosnia and Herzegovina is the central part of the natural southern prevalence of pedunculate oak (*Quercus robur*, L.), and as such, it has a specific genetic structure in comparison to the central and northern part of the areal (Ballian, Belletti, Ferrazzini, Bogunić & Kajba, 2010). This species and its distribution in Bosnia and Herzegovina make a specific connection between southern and eastern provenances of the Balkan Peninsula on the one side and provenances from Central Europe on the other side. It has a significant role in the circulation of genes from south to north and vice versa, and from west to east and vice versa (Slade, Škvorc, Ballian, Gračan & Papeš, 2008).

According to the data of state forest inventory in Bosnia and Herzegovina 1964-1968 (Matić et al., 1971), surface of high forests of less present species is 32,369 ha, where approximately 31.7% or 10,261 ha are pedunculate oak forests. Unlike Matić et al. (1971), Klepac (1988) states that total pedunculate oak surface in BiH in that period was 30,000 ha. According to estimates, close to 5% of Bosnia and Herzegovina's surface are plains favorable for the development of pedunculate oak. Forests of pedunculate oak and common hornbeam (*Carpinus betulus – Quercetum roboris*) as potential vegetation according to ecological and vegetation zoning (Stefanović, Beus, Burlica, Dizdarević & Vukorep, 1983), are shown in Figure 1.

Memišević Hodžić (2015), Ballian and Memišević Hodžić (2016) and Memišević Hodžić and Ballian (2016) researched morphological characteristics of pedunculate oak (height and root collar diameter) in Bosnian-Herzegovinian provenance test in Žepče. Results showed statistically significant differences between analyzed provenances through variance analysis, which was confirmed by the Duncan test. The research included 28 populations/provenances.

Memišević Hodžić (2015) researched genetic variability of pedunculate oak in Žepče provenance test using isoenzyme markers. Most of fourteen analyzed loci were polymorphic. Results showed differences between analysed populations, especially between small, anthropogenically changed populations on the one side and large populations on the other side.

Due to their specific genetic structure, pedunculate oak populations in Bosnia and Herzegovina are important for preserving diversity of this species in Europe.

For realizing the level of variability of pedunculate oak in Bosnia and Herzegovina we established, provenance test in Žepče. Provenance test will represent preservation by
We measured height and root collar diameter in four-year-old, six-year-old, and eight-year-old plants on all plants of all provenances. Data were processed using SPSS 20.0 statistics package, resulting in the following parameters:

- Descriptive statistical indicators (mean value, standard deviation, and standard error)
- Variance analysis for determining intrapopulation and interpopulation variability of analyzed characteristics
- Duncan test for determining provenance grouping by analysed characteristics

The second part of the research were genetic and molecular analyses using biochemical markers. We chose 20 specific provenances and analyzed fifty plants from each of them. We used isoenzymes as biochemical markers. A set of ten isoenzyme systems with a total of 14 gene loci were analyzed (Table 2).

We used isoenzyme analysis procedure based on previously confirmed methods by Cheliak and Pitel (1984) and Konnert, Fromm & Wimmer (2004). For calculation the fixation index we used SAS (Statistical Analysis System) program (MACGEN – Stauber & Hertel, 1997).

Table 1. List of analysed provenances

| No. | Provenance          | Latitude | Longitude | Altitude | Morphological analysis | Molecular (isoenzyme) analysis |
|-----|---------------------|----------|-----------|----------|------------------------|-------------------------------|
| 1   | Bijeljina           | 44º 43' 50" | 19º 13' 30" | 93       | yes                    | yes                           |
| 2   | Bosanska Dubica     | 45º 06' 24" | 16º 40' 32" | 145      | yes                    | yes                           |
| 3   | Bosanska Gradiška   | 45º 06' 64" | 17º 18' 63" | 91       | yes                    | yes                           |
| 4   | Bosanski Brod       | 45º 05' 27" | 18º 00' 38" | 84       | yes                    | yes                           |
| 5   | Bosansko Grabovo    | 44º 01' 05" | 16º 38' 24" | 703      | yes                    | yes                           |
| 6   | Bugojno             | 44º 06' 00" | 17º 26' 31" | 537      | yes                    | yes                           |
| 7   | Drvar               | 44º 23' 39" | 16º 21' 54" | 462      | yes                    | yes                           |
| 8   | Hrgovi Srrebrenik   | 44º 49' 06" | 18º 34' 11" | 133      | yes                    | yes                           |
| 9   | Jelah               | 44º 39' 09" | 17º 56' 46" | 181      | yes                    | yes                           |
| 10  | Kaćuni              | 44º 03' 59" | 17º 56' 13" | 443      | yes                    | yes                           |
| 11  | Kiseljak            | 43º 56' 30" | 18º 04' 56" | 477      | yes                    | no                            |
| 12  | Ključ               | 44º 30' 56" | 16º 48' 42" | 260      | yes                    | yes                           |
| 13  | Knežina             | 44º 01' 40" | 18º 44' 53" | 759      | yes                    | no                            |
| 14  | Kotor Varoš         | 44º 39' 07" | 17º 21' 35" | 252      | yes                    | yes                           |
| 15  | Lukavica            | 43º 49' 26" | 18º 21' 58" | 552      | yes                    | no                            |
| 16  | Miljevina Foča      | 43º 31' 06" | 18º 38' 56" | 627      | yes                    | yes                           |
| 17  | Mrk. Grad           | 44º 27' 04" | 16º 58' 42" | 753      | yes                    | yes                           |
| 18  | Mutnica             | 44º 58' 55" | 15º 50' 54" | 270      | yes                    | yes                           |
| 19  | Nević Polje         | 44º 11' 46" | 17º 42' 11" | 476      | yes                    | no                            |
Table 3. Results of plant height

As shown in Table 3, the average height of all four-year-old plants in provenance test amounted to 50.3 cm, 117.9 cm of six-year-old plants, and 168.7 cm of eight-year-old plants.

Miljevina Foča provenance had the lowest average height in four-year-old plants with 38.8 cm, Stojčevac provenance in six-year-old plants with 90.8 cm, and Visoko Muhašinovići provenance in eight-year-old plants with 123.6 cm.

Jelah provenance had the maximum average height of plants of all three age groups with 74.3 cm, 152.1 cm, and 219.0 cm.

Table 3. Descriptive indicators for plant height per provenances and per age of plants

| Provenance         | No. | Mean (cm) | Std. dev. (cm) | Std. err. | Mean (cm) | Std. dev. (cm) | Std. err. | Mean (cm) | Std. dev. (cm) | Std. err. |
|-------------------|-----|-----------|----------------|-----------|-----------|----------------|-----------|-----------|----------------|-----------|
| Bijeljina         | 99  | 48.5      | 21.1           | 2.1       | 122.7     | 47.3           | 5.0       | 183.5     | 76.5           | 8.1       |
| Bos.Dubica        | 102 | 55.7      | 26.2           | 2.5       | 133.9     | 52.7           | 5.2       | 194.5     | 77.5           | 7.7       |
| Bos.Gradiska      | 101 | 41.7      | 17.3           | 1.7       | 102.3     | 33.4           | 3.5       | 151.8     | 59.3           | 6.3       |
| Bos.Brod          | 82  | 53.7      | 19.2           | 2.1       | 134.8     | 30.3           | 3.7       | 198.6     | 55.5           | 6.8       |
| Bos.Grahamo       | 92  | 50.7      | 17.9           | 1.9       | 112.8     | 33.9           | 3.8       | 149.4     | 50.8           | 5.8       |
| Bugojno           | 95  | 56.9      | 20.7           | 2.1       | 129.3     | 40.5           | 4.4       | 188.1     | 78.5           | 8.7       |
| Drvar             | 58  | 63.9      | 30.8           | 4.0       | 138.1     | 44.5           | 5.9       | 209.8     | 68.7           | 9.4       |
| Hrgovi Sreb.      | 96  | 48.0      | 21.5           | 2.2       | 123.7     | 42.0           | 4.4       | 178.8     | 61.2           | 6.4       |
| Jelah             | 101 | 74.3      | 41.9           | 4.2       | 152.1     | 66.3           | 6.6       | 219.0     | 97.6           | 9.8       |
| Kačuni            | 88  | 53.1      | 23.2           | 2.5       | 141.1     | 46.4           | 5.3       | 212.0     | 79.6           | 9.1       |
| Kiseljak          | 102 | 45.1      | 17.0           | 1.7       | 95.5      | 41.5           | 4.1       | 144.8     | 55.6           | 5.6       |
| Ključ             | 80  | 45.6      | 20.4           | 2.3       | 112.0     | 47.1           | 5.9       | 159.1     | 71.4           | 9.2       |
Table 3 (continued)

| Provenance     | No. | four-year-old | six-year-old | eight-year-old |
|----------------|-----|---------------|--------------|----------------|
|                |     | Mean (cm) | Std. (cm) | Std. err. | Mean (cm) | Std. (cm) | Std. err. | Mean (cm) | Std. (cm) | Std. err. |
| Knežina        | 93  | 49.4 | 24.5 | 2.5 | 114.6 | 48.3 | 5.3 | 154.1 | 61.8 | 6.9 |
| Kotor Varoš    | 99  | 46.5 | 16.8 | 1.7 | 104.1 | 37.2 | 3.9 | 161.8 | 60.0 | 6.4 |
| Lukavica       | 83  | 47.4 | 20.4 | 2.2 | 100.9 | 31.0 | 3.7 | 136.4 | 49.1 | 6.3 |
| Miljevina Foča | 89  | 38.8 | 17.6 | 1.9 | 92.7 | 33.8 | 3.9 | 135.7 | 53.1 | 6.1 |
| Mrk. Grad      | 90  | 44.6 | 16.5 | 1.7 | 112.7 | 33.1 | 3.7 | 152.9 | 55.0 | 6.4 |
| Mutnica        | 58  | 51.7 | 19.9 | 2.6 | 140.6 | 47.8 | 7.0 | 209.0 | 60.1 | 8.9 |
| Nević Polje    | 76  | 49.9 | 26.9 | 3.1 | 110.2 | 43.8 | 5.5 | 149.8 | 70.2 | 9.0 |
| Novi Šeher     | 86  | 50.0 | 26.0 | 2.8 | 116.1 | 45.8 | 5.4 | 151.0 | 67.7 | 8.4 |
| Olovo          | 99  | 48.4 | 16.3 | 1.6 | 107.3 | 30.8 | 3.2 | 150.1 | 60.4 | 6.6 |
| Sokolac        | 89  | 51.5 | 24.6 | 2.7 | 128.5 | 51.4 | 6.6 | 185.1 | 78.0 | 10.2 |
| Stojevac       | 90  | 39.3 | 16.2 | 1.7 | 90.8 | 33.9 | 3.9 | 127.0 | 48.7 | 5.6 |
| Vinac          | 83  | 48.4 | 24.6 | 2.7 | 128.5 | 51.4 | 6.6 | 185.1 | 78.0 | 10.2 |
| Visoko Muhaš.  | 95  | 45.5 | 21.1 | 2.2 | 97.4 | 33.2 | 3.5 | 123.6 | 49.2 | 5.4 |
| Zavidovići     | 96  | 49.3 | 20.6 | 2.1 | 118.0 | 36.7 | 4.0 | 173.7 | 67.2 | 7.3 |
| Zepče          | 100 | 57.3 | 23.1 | 2.3 | 124.9 | 45.1 | 4.6 | 176.3 | 67.5 | 7.0 |
| Živinice        | 94  | 55.0 | 27.8 | 2.9 | 133.5 | 53.0 | 5.8 | 195.0 | 84.9 | 9.4 |
| Total          | 2516 | 50.3 | 23.7 | 0.5 | 117.9 | 45.6 | 1.0 | 168.7 | 71.5 | 1.5 |

The Duncan test for plant height showed grouping of provenances in 10 groups at four-year-old plants, in 16 groups at six-year-old plants, and in 13 groups at eight-year-old plants. Considering that groups overlapped, it is not possible to determine with certainty which provenances show more permanent grouping at this age.

Variance analysis for plant height shows that there are statistically significant differences between provenances in plants of all analysed ages (F izr > F tab; Sig. < 0.05), Table 4.

Table 4. Analysis of variance for plant height per age of plants

| Age of plants | Source of variation | Sum of squares | Degrees of freedom | Mean square | F | F-tab | Significance |
|---------------|---------------------|----------------|--------------------|-------------|---|-------|--------------|
| four-year-old | Among Provenances   | 128438.5       | 27                 | 4757.0      | 9.2* | 1.49 | 0.0          |
|               | Within Provenances  | 1285172.9      | 2488               | 516.6       |    |       |              |
|               | Total               | 1413611.4      | 2515               |             |    |       |              |
| six-year-old  | Among Provenances   | 572766.9       | 27                 | 21213.6     | 11.5* | 1.49 | 0.0          |
|               | Within Provenances  | 4083455.1      | 2488               | 1841.9      |    |       |              |
|               | Total               | 4656222.0      | 2515               |             |    |       |              |
| eight-year-old| Among Provenances   | 1505865.04     | 27                 | 55772.78    | 12.49* | 1.49 | 0.00         |
|               | Within Provenances  | 9572090.29     | 2488               | 4466.68     |    |       |              |
|               | Total               | 11077955.33    | 2515               |             |    |       |              |
Results of diameter of root collar

Average value of root collar diameter of all four-year-old plants in provenance tests amounted to 13.1 mm, in six-year-old plants 28.9 mm, and in eight-year-old plants 42.8 mm (Table 5).

Vinac provenance had the lowest average root collar diameter in four-year-old plants with 11.3 mm, Stojčevac provenance in six-year-old plants with 23.7 mm, and Visoko Muhašinići provenance in eight-year-old plants with 33.0 mm. Provenance Jelah had the biggest average root collar diameter in four-year-old plants, 16.2 mm, Mutnica Cazin in six-year-old plants, 36.2 mm, and Jelah in eight-year-old plants, 53.3 mm.

Table 5. Descriptive indicators for root collar diameter per provenances and per age of plants

| Provenance          | No. | Mean (cm) | Std. dev. (cm) | Std. error | Mean (cm) | Std. dev. (cm) | Std. error | Mean (cm) | Std. dev. (cm) | Std. error |
|---------------------|-----|-----------|----------------|------------|-----------|----------------|------------|-----------|----------------|------------|
| Bijeljina           | 99  | 14.5      | 5.1            | 0.5        | 31.1      | 10.8           | 1.1        | 45.7      | 17.4           | 1.8        |
| Bos.Dubica          | 102 | 14.2      | 5.2            | 0.5        | 32.7      | 12.3           | 1.2        | 48.7      | 20.5           | 2.0        |
| Bos.Gradiška        | 101 | 12.4      | 4.6            | 0.5        | 26.1      | 7.7            | 0.8        | 40.0      | 16.7           | 1.8        |
| Bos.Brod            | 82  | 13.4      | 4.0            | 0.4        | 31.6      | 6.6            | 0.8        | 47.6      | 13.9           | 1.7        |
| Bos.Grahovo         | 92  | 14.1      | 4.5            | 0.5        | 28.7      | 6.8            | 0.8        | 39.1      | 13.0           | 1.4        |
| Bugojno             | 95  | 12.9      | 4.5            | 0.5        | 29.7      | 7.8            | 0.9        | 43.7      | 16.5           | 1.8        |
| Drvar               | 58  | 15.6      | 4.9            | 0.6        | 33.9      | 11.8           | 1.6        | 52.8      | 18.2           | 2.5        |
| Hrgovi Srebrenik     | 96  | 12.9      | 4.3            | 0.4        | 26.7      | 6.7            | 0.7        | 42.5      | 13.2           | 1.4        |
| Jelah               | 101 | 16.2      | 6.2            | 0.6        | 34.7      | 14.0           | 1.4        | 53.3      | 23.9           | 2.4        |
| Kačuni              | 88  | 14.9      | 5.0            | 0.5        | 30.8      | 8.7            | 1.0        | 45.9      | 18.5           | 2.1        |
| Kiseljak            | 102 | 12.9      | 3.8            | 0.4        | 27.2      | 8.9            | 0.9        | 40.2      | 15.8           | 1.6        |
| Ključ               | 80  | 11.6      | 4.1            | 0.5        | 27.4      | 10.1           | 1.3        | 40.7      | 21.9           | 2.8        |
| Knežina             | 93  | 11.4      | 4.5            | 0.5        | 27.4      | 8.9            | 1.0        | 39.3      | 17.8           | 2.0        |
| Kotor Varoš         | 99  | 13.6      | 4.7            | 0.5        | 28.0      | 7.9            | 0.8        | 42.7      | 16.1           | 1.7        |
| Lukavica            | 83  | 13.2      | 5.1            | 0.6        | 25.5      | 7.0            | 0.8        | 37.8      | 13.6           | 1.8        |
| Miljevina Foča      | 89  | 11.7      | 4.3            | 0.5        | 25.6      | 7.6            | 0.9        | 34.6      | 12.8           | 1.5        |
| Mrk.Grad            | 90  | 12.3      | 4.0            | 0.4        | 27.9      | 6.5            | 0.7        | 39.3      | 14.2           | 1.7        |
| Mutnica Cazin       | 58  | 14.7      | 4.2            | 0.5        | 36.2      | 10.3           | 1.5        | 52.6      | 17.9           | 2.6        |
| Nević Polje         | 76  | 12.3      | 4.5            | 0.5        | 27.3      | 8.1            | 1.0        | 39.4      | 15.8           | 2.0        |
| Novi Šeher          | 86  | 12.9      | 4.7            | 0.5        | 27.8      | 8.2            | 1.0        | 41.6      | 16.5           | 2.0        |
| Olovo               | 99  | 13.4      | 4.1            | 0.4        | 27.8      | 7.5            | 0.8        | 42.0      | 16.6           | 1.8        |
| Sokolac             | 89  | 11.9      | 3.7            | 0.4        | 27.6      | 8.2            | 0.9        | 40.1      | 16.7           | 1.9        |
| Stojčevac           | 90  | 11.5      | 3.3            | 0.4        | 23.7      | 5.2            | 0.6        | 33.1      | 10.9           | 1.2        |
| Vinac               | 83  | 11.3      | 4.1            | 0.5        | 32.0      | 11.7           | 1.5        | 49.4      | 21.9           | 2.9        |
| Visoko Muhaš.       | 95  | 12.4      | 3.9            | 0.4        | 25.3      | 7.7            | 0.8        | 33.0      | 14.1           | 1.5        |
| Zavidovići          | 96  | 12.1      | 4.2            | 0.4        | 28.3      | 8.0            | 0.9        | 42.2      | 15.6           | 1.7        |
| Žepce               | 100 | 13.4      | 4.5            | 0.4        | 29.8      | 9.5            | 1.0        | 45.0      | 17.1           | 1.8        |
| Živinice             | 94  | 13.6      | 5.0            | 0.5        | 31.4      | 11.7           | 1.3        | 48.1      | 19.9           | 2.2        |
| Total               | 2516| 13.1      | 4.7            | 0.1        | 28.9      | 9.5            | 0.2        | 42.8      | 17.7           | 0.4        |

The Duncan test for root collar diameter showed grouping of provenances in 10 groups at four-year-old and six-year-old, and in nine groups at eight-year-old. Groups overlapped

Variance analysis showed that there are statistically significant differences between provenances in terms of root collar diameter in plants of all analyzed ages (F izr > F tab; Sig. < 0.05), Table 6.
Table 6. Analysis of variance of diameter of root collar per age of plants

| Age of plants | Source of variation | Sum of squares | Degrees of freedom | Mean square | F    | F-tab | Significance |
|---------------|---------------------|----------------|--------------------|-------------|------|-------|--------------|
| four-year-old| Between Provenances | 3821.9         | 27                 | 141.5       | 6.9* | 1.49  | 0.0          |
|               | Within Provenances  | 50750.1        | 2488               | 20.4        |      |       |              |
|               | Total               | 54572.0        | 2515               |             |      |       |              |
| six-year-old  | Between Provenances | 18485.1        | 27                 | 684.6       | 8.3* | 1.49  | 0.0          |
|               | Within Provenances  | 181974.7       | 2488               | 82.1        |      |       |              |
|               | Total               | 200459.8       | 2515               |             |      |       |              |
| eight-year-old| Between Provenances | 1505865.0      | 27                 | 55772.8     | 12.5*| 1.49  | 0.00         |
|               | Within Provenances  | 9572090.3      | 2488               | 4466.7      |      |       |              |
|               | Total               | 11077955.3     | 2515               |             |      |       |              |

Results of isoenzyme analysis - Fixation index (Wright’s inbreeding coefficient)

Fixation index average value for all provenances was positive but low, 0.0033.

Eight out of twenty provenances showed positive fixation index mean values, which means that there is inbreeding. Those provenances are: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad and Sokolac (Table 7).

Bosanska Dubica provenance had the highest positive fixation index mean value with 0.1019 and positive values in five gene loci. Provenances Drvar and Miljevina had positive fixation index values in five gene loci as well.

Olovo provenance had the lowest negative fixation index mean value, -0.1269. Fixation index values for this provenance were negative for all loci except for PGM-B. Fixation index values of 0.0000 in certain provenances and gene loci showed a balanced state in those provenances and gene loci according to Hardy-Weinberg.
| Provenance         | FEST  | FEST  | ADH   | AAP   | PGM   | 6PGDH  | 6PGDH  | IDH   | IDH   | IDH   | PGI   | GOT   | MNR   | SDH   | Mean Fi |
|--------------------|-------|-------|-------|-------|-------|--------|--------|-------|-------|-------|-------|-------|-------|-------|---------|
| Bijeljina          | 0.182 | -0.030| -0.007| 0.143 | 0.043 | -0.064 | -0.010 | -0.070| 0.193 | 0.006 | 0.000 | -0.024| 0.116 | -0.031| 0.032   |
| Bos. Dubica        | 0.608 | -0.044| 0.000 | 0.317 | 0.791 | -0.053 | 0.000  | -0.224| 0.062 | 0.046 | -0.010| -0.024| 0.031 | -0.010| 0.102   |
| Bos. Gradiška      | -0.010| -0.056| -0.111| 0.047 | 0.392 | 0.000  | -0.020| -0.131| 0.045 | -0.180| -0.031| -0.055| -0.076| -0.015| -0.014  |
| Bos. Brod          | 0.298 | -0.077| -0.226| 0.217 | 0.083 | -0.090 | -0.010| -0.141| 0.000 | 0.072 | -0.010| -0.030| 0.065 | 0.000 | -0.010  |
| Bos. Grahamo       | 0.373 | -0.080| -0.070| -0.052| 0.410 | -0.040 | 0.000  | -0.031| -0.124| -0.114| 0.000 | -0.081| 0.792 | 0.000 | 0.070   |
| Bugojno            | 0.000 | -0.140| -0.176| 0.187 | 0.070 | 0.000  | -0.020| 0.145 | 0.042 | 0.030 | 0.053 | -0.087| -0.010| -0.010| -0.018  |
| Drvar              | -0.137| -0.047| -0.054| 0.170 | 0.033 | -0.020| -0.020| -0.122| -0.083| 0.083 | 0.000 | 0.149 | 0.412 | 1.000 | 0.093   |
| Hrgovi Srebr.      | -0.034| -0.031| -0.143| 0.004 | 0.163 | -0.064| -0.053| 0.111 | -0.244| 0.242 | 0.000 | -0.035| 0.120 | -0.156| -0.009  |
| Jelah              | 0.368 | -0.042| -0.180| 0.092 | 0.281 | -0.250| 0.000  | -0.152| -0.091| 0.218 | -0.031| -0.010| 0.249 | -0.338| 0.009   |
| Kacuni             | -0.055| -0.096| 0.238 | 0.023 | -0.122| -0.020| 0.166  | 0.124 | -0.099| 0.108 | -0.075| -0.015| -0.012| -0.042| -0.015  |
| Kluč              | -0.010| -0.054| -0.050| 0.126 | 0.290 | -0.010| -0.010| 0.250 | -0.147| -0.240| 0.053 | -0.037| -0.134| -0.042| -0.001  |
| Kotor Varaš        | -0.025| 0.000 | 0.085 | 0.060 | 0.368 | 0.000 | 0.000  | -0.012| 0.071 | -0.087| -0.177| -0.159| -0.283| -0.010| -0.024  |
| Miljevina          | 0.341 | -0.114| -0.111| 0.266 | 0.344 | -0.010| 0.000  | -0.177| 0.077 | 0.344 | 0.059 | -0.080| 0.209 | 0.015 | 0.081   |
| Mrkonjić Gr.       | -0.077| 0.000 | -0.047| 0.257 | 0.025 | -0.055| 0.000  | -0.106| 0.039 | -0.102| -0.053| -0.015| 0.363 | 0.000 | 0.016   |
| Mutnica            | -0.025| 0.042 | -0.030| 0.170 | 0.190 | 0.064 | 0.000  | -0.034| -0.204| -0.085| -0.024| -0.020| -0.033| -0.010| -0.015  |
| Okovo              | -0.061| -0.024| -0.023| 0.360 | 0.012 | -0.010| -0.099| -0.069| -0.346| -0.191| 0.000 | 0.000  | 0.045 | -0.111| -0.127  |
| Sokolac            | 0.425 | -0.056| -0.007| 0.146 | 0.154 | -0.136| -0.010| -0.073| 0.048 | 0.039 | -0.047| -0.010| -0.073| -0.053| 0.003   |
| Stojčevac          | -0.040| 0.000 | -0.132| -0.330| -0.005| 0.000 | 0.000  | -0.065| -0.426| -0.120| 0.368 | -0.024| -0.010| -0.010| -0.057  |
| Žepče              | -0.059| -0.100| 0.138 | 0.007 | 0.034 | 0.000 | 0.000  | -0.042| 0.062 | -0.463| -0.064| -0.010| -0.034| -0.034| -0.020  |
| Žižnica            | -0.044| -0.100| -0.118| 0.203 | 0.072 | -0.075| 0.117 | 0.024 | 0.250 | 0.077 | -0.024| -0.053| -0.068| 0.336 | -0.003  |
| Fr for g. loci     | 0.101 | -0.057| -0.060| 0.085 | 0.137 | -0.048| -0.018| -0.035| -0.099| 0.004 | -0.003| -0.032| 0.047 | 0.023 | 0.003   |
Discussion

Pedunculate oak provenance test in Žepče is the first test for pedunculate oak research in Bosnia and Herzegovina. Unlike Bosnia and Herzegovina, there were pedunculate oak provenance tests in Europe 80-100 years ago (Hauch, 1909; Cieslar, 1923). In neighboring Croatia, researches were started in 1988 (Graćan, 1995; Graćan, 1996) and later continued (Popović, Ivanković & Bogdan, 2014). Reasons for low interest for pedunculate oak in Bosnia and Herzegovina are its small distribution and fragmentation of populations, which is a direct consequence of high exploitation of this species in the period from 1839 to 1914 (Begović, 1960; Begović, 1978; Memišević, 2008). Morphologic analyses included measuring plant height and root collar diameter in four-year-old, six-year-old and eight-year-old plants in the Bosnian-Herzegovinian pedunculate oak provenance test. This research, as well as previous researches, (Cieslar, 1923; Graćan, 1995; Roth, 2003; Roth, 2006), showed statistically significant differences among provenances for all analyzed morphologic characteristics. Relation of juxtaposition of certain provenances in terms of morphological characteristics has not significantly changed since the establishment of the provenance test (Table 3, Table 5).

Fixation index (Fst) indicates inbreeding existence in populations – negative fixation index values show a lack of inbreeding, while positive values indicate the presence of inbreeding (Bergmann, Gregorius & Larsen, 1990). This index shows the deviation level of real heterozygosity from the expected Hardy Weinberg balance (Morgenstern, 1996).

Fixation index average value for all provenances was positive but low, 0.0033, which means that Bosnia and Herzegovina populations show low intrapopulation diversity, as a consequence of significant fragmentation of pedunculate oak.

Bosanska Dubica provenance had the highest mean fixation index value, 0.1019 and positive values in five gene loci, as well as populations of Drvar and Miljevina. Even though Bosanska Dubica population belongs to the region of Posavina and it is open towards pedunculate oak populations in Croatia, it is small and under the constant anthropogenic influence, while Drvar and Miljevina populations are small and isolated. Under the effect of genetic drift, specific processes dominate those small and isolated populations, which indicate possible presence of inbreeding. However, even with high fixation index values, provenances of Bosanska Dubica and Drvar show good growth in the provenance test, unlike Miljevina provenance.

According to Ballian (2005), the obtained fixation index values are good indicators which cultivation and economic measures need to be taken. Even if we did all necessary measures, it often happens that there is no natural regeneration in forests or it is incomplete. Breeders mostly attribute this to factors of climate, land, etc. However, the problem is often in the genetic structure of forest trees and reproductive relations within forest trees population. In the past, genetic burden researches were time-consuming and required series of field tests and seed analyses. Today we can relatively quickly and in a scientifically accepted manner, estimate the genetic burden of a population using the obtained fixation index values.

Olovo provenance had the lowest fixation index value, -0.1269, and negative values for all loci except for PGM-B. This results were not expected considering that Olovo population is small and isolated. Olovo population shows good growth which corresponds to a low value of fixation index, suggesting a good genetic structure.

Jelah population showed the best growth among eight-year-old plants, and it has relatively high fixation index value.

Interventions in populations which have high positive fixation index values should be done carefully. To avoid violating the already unstable structure of these populations we should exclude huge interventions. The number of seed trees for the rehabilitation process should be far higher than in populations with a low fixation index value.

If genetic drift is present in a population, it is enough to exclude only a few individuals from reproduction process to immediately get visible and inestimable consequences in the genetic structure of the future population. Participation of empty seeds will increase, as well as general weakness and depressiveness
in the growth of natural offspring in the rehabilitation process. Based on the obtained research results, we should give special attention to Bosanska Dubica, Drvar, and Miljevina populations. During rehabilitation of these populations, it is necessary to conduct molecular and genetic analyses and direct cultivation and economic activities towards the increase of heterozygosity.

Conclusion
Based on the results of research of morphologic characteristics of pedunculate oak and fixation index in provenance test in Bosnia and Herzegovina we can conclude the following:

1. All analyzed morphologic characteristics showed in variance analysis that there are statistically significant differences among analysed provenances, which was confirmed by the Duncan test.

2. Mean height of all four-year-old plants in provenance test was 50.3 cm, six-year-old 117.9 cm, and eight-year-old 168.7 cm.

3. Miljevina Foča provenance had the lowest mean height of four-year-old plants, 38.8 cm, while Jelah provenance had the maximum mean height, 74.3 cm. Stojčevac provenance had the lowest mean height of six-year-old plants, 90.8 cm, while Jelah provenance had the maximum mean height, 152.1 cm. Visoko Muhašinovići provenance had the lowest mean height, 123.6 cm, while Jelah provenance had the maximum mean height, 219.0 cm.

4. Mean root collar diameter in all four-year-old plants was 13.1 mm, in six-year-old plants 28.9 mm, and in eight-year-old plants 42.8 mm.

5. Vinac provenance had the lowest mean root collar diameter in four-year-old plants, 11.3 mm, Stojčevac provenance in six-year-old plants, 23.7 mm, and Visoko Muhašinovići provenance in eight-year-old plants, 33.0 mm. Jelah provenance had the maximum root collar diameter in four-year-old plants, 16.2 mm, Mutnica Cazin provenance in six-year-old plants, 36.2 mm and Jelah provenance in eight-year-old plants, 53.3 mm.

6. Provenances of Miljevina Foča, Stojčevac and Visoko Muhašinovići are at the bottom of the list with lowest growth of four-, six-, and eight-year-old plants. Provenances of Drvar, Mutnica Cazin, Kačuni, and Jelah showed the best growth.

7. Eight out of twenty provenances showed good positive fixation index values, especially Miljevina Foča, Bosanska Dubica, and Drvar provenances. We should give special attention during management and silvicultural measures in these populations. Reproductive material from these provenances should not be used in rehabilitation of pedunculate oak provenances in Bosnia and Herzegovina, considering their fixation index values, even if they have good growth.

8. Positive fixation index value of Miljevina provenance matches its low growth, while this is not the case in Drvar and Bosanska Dubica provenances.

9. Olovo provenance, even though it is small and isolated, shows good growth, which corresponds to its low fixation index value which shows good genetic structure.

10. We should use the obtained results while planning measures of protection and reintroduction of pedunculate oak in Bosnia and Herzegovina as well as management activities in the remaining population.

11. It is necessary to continue research because the results obtained in the early, juvenile stage are incomplete and burdened with numerous flaws.

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