Kastamonu Uni., Orman Fakültesi Dergisi, 2019, 19 (2):186-196 Research Article
Kastamonu Univ., Journal of Forestry Faculty
Doi:10.17475/kastorman.626373

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

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Received Date: 27.12.2018 Accepted Date: 29.04.2019

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 Denemesinin 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 araştırmanın amacı, orijinlerin büyüme ve fiksasyon indeksi arasındaki ilişkiyi 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 ölçülmüştür.

Sonuçlar: Orijin denemesindeki bütün bitkilerin ortalama 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 ortalama 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 anlamlı farklılıklar tespit edilmiştir. Miljevina Foča, Stojčevac, ve Visoko Muhašinovići orijinlerinin 4, 6 ve 8 yıllık fidanları en düşük büyümeye sahipken, Drvar, Mutnica Cazin, Kaćuni, ve Jelah orijinleri en iyi gelişmeyi göstermiştir.

Önemli vurgular: Yirmi sekiz orijinden 8'i, pozitif fiksasyon indeksi değerleri göstermiştir. Özellikle Miljevina Foča, Bosanska Dubica ve Drvar orijinleri vurgulanması gereken pozitif fiksasyon endeksi değerlerine sahiptir. Miljevina Foča orijini örneğinde; fiksasyon indeksi zayıf büyüme ile eşleşirken, Drvar ve Bosanska Dubica durum farklıdır.

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

Citation (Atıf): Hodžić, M.M. & Ballian, D. (2019). Growth Tendency of *Quercus robur* L. Provenances in Bosnia and Herzegovina Provenance Test with Relation to Fixation Index. *Kastamonu University Journal of Forestry Faculty*, 19 (2), 186-196.

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 (*Carpino betuli – Quercetum roboris*) as potential vegetation according to ecological and vegetation zoning (Stefanović, Beus, Burlica, Dizdarević & Vukorep, 1983), are shown in Figure 1.

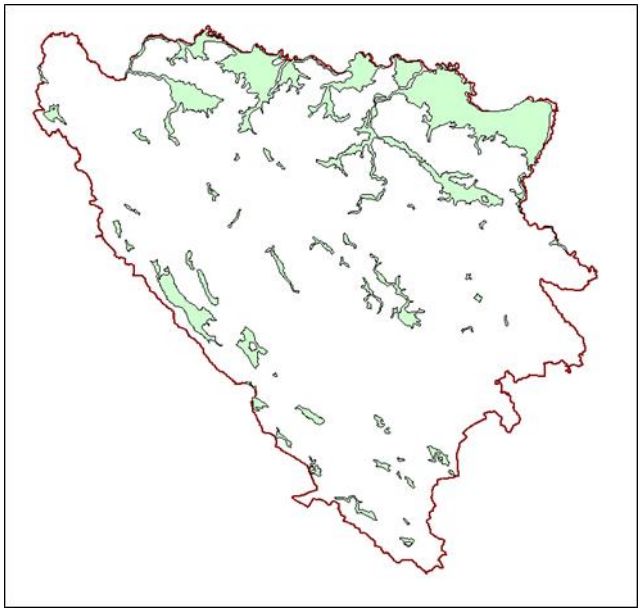

Figure 1. Forests of pedunculate oak and common hornbeam as potential vegetation in B&H

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

ex situ method and provide plenty of data required for preservation using *in situ* methods.

The research aim is to determine variability within and among pedunculate oak provenances by measuring morphologic characteristics: height and root collar diameter in four-year-old, six-year-old, and eight-year-old plants, and comparing with fixation index values obtained through isoenzyme analyses.

Material and Methods

During 2007 we collected seed material of pedunculate oak in 28 locations – populations (Table 1) for establishing provenance test. Seeding in Dunemann rows in Žepče-Lugovi nursery, according to a previously fixed order, was conducted in autumn 2007. Plants were 1year old. They were planted into the field test in spring 2009, using a classic method – planting in 30 cm deep holes, with 2 m x 2 m distance between plants. According to the scheme, we planted per 36 plants from each provenance in three blocks (3 repetitions). We made a protection belt around each block with two rows of seedlings for reducing edge effects to the test surface.

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 calculationg 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	Morpho-logical 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 Grahovo	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 Srebrenik	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 1 (continued)

20	Novi Šeher	44º 07' 44"	18º 36' 11"	542	yes	no
21	Olovo	43º 55' 17"	18º 48' 53"	866	yes	yes
22	Sokolac	43º 48' 40"	18º 17' 25"	506	yes	yes
23	Stojčevac	44º 15' 48"	17º 17' 08"	408	yes	yes
24	Vinac	44º 00' 38"	18º 08' 45"	413	yes	no
25	Visoko Muhašinovići	44º 26' 07"	18º 07' 49"	210	yes	no
26	Zavidovići	44º 25' 01"	19º 07' 22"	141	yes	no
27	Žepče	44º 25' 35"	18º 03' 10"	224	yes	yes
28	Živinice	44º 27' 58"	18º 41' 09"	216	yes	yes

Table 2. Analysed enzyme systems and gene locus

No	Enzyme system	Gene locus	Investigated alleles
1.	Alaninaminopeptidase	AAP-A	A_2, A_4, A_5, B_3, B_5
2.	Aspartataminotransferaze	GOT-B	B_3, C_4
3.	Fluoreszierende Esterase	FEST-A, FEST-C	B_4
4.	Alkoholdehydrogenase	ADH-A	A_2, A_3
5.	Isocitratdehydrogenase	IDH-A, IDH-B, IDH-C	A_4, B_2, B_5
6.	Menadionreduktase	MNR-A	A_3
7.	6-Phosphogluconatdehydrogenase	6-PDGH-A, 6-PDGH-B	B_3
8.	Phosphoglucose-isomerase	PGI-B	A_3, B_4
9.	Phosphoglucomutase	PGM-B	A_3, B_2, B_3
10.	Shikimatdehydrogenase	SKDH-A	A_3

Results

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.	four-year-old			six-year-old			eight-year-old		
		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.Grahovo	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
Kaćuni	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. dev. (cm)	Std. err.	Mean (cm)	Std. dev. (cm)	Std. err.	Mean (cm)	Std. dev. (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	24.1	2.6	121.0	43.4	4.8	159.0	63.3	7.2
Stojčevac	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
Žepče	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 Muhašinovići 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.	four-year-old			six-year-old			eight-year-old		
		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
Kaćuni	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.

Table 7. Fixation index (Fi)

Provenance	Gene locus														Mean Fi
	FEST -A	FEST -C	ADH -A	AAP -A	PGM -B	6PGDH -A	6PGDH -B	IDH -A	IDH -B	IDH -C	PGI -B	GOT -B	MNR -A	SDH -A	
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. Grahovo	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
Kaćuni	-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
Ključ	-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 Varoš	-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
Olovo	-0.061	-0.024	-0.023	-0.360	0.012	-0.010	-0.099	-0.069	-0.346	-0.191	0.000	0.000	-0.495	-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	-0.042	0.062	-0.463	-0.064	-0.010	-0.034	-0.034	-0.020	-0.047
Živinice	-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
F_I 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 (Gračan, 1995; Gračan, 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 andeight-year-old plants in the Bosnian-Herzegovinian pedunculate oak provenance test. This research, as well as previous researches, (Cieslar, 1923; Gračan, 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 vallues 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, Kaćuni, 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 reamining 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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