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Pedunculate oak (*Quercus robur* L.) in Bosnia and Herzegovina - state and perspectives

Bosna Hersek'teki saplı meşe ormanlarının değerlendirilmesi

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Abstract

Bosnia and Herzegovina is located in Southeast Europe, and more than half its area of 51.129 km² belongs to forests and forest lands. 93% of the forests of Bosnia and Herzegovina are natural forests and have many plant species, many of which are endemic. There are eight oak species growing in Bosnia and Herzegovina. The most important ones are *Quercus robur* (despite being almost extinct due to over-exploitation during the late XIX and early XX century), and *Quercus petraea*. Pedunculate oak in Bosnia and Herzegovina is found in mixed stands with common hornbeam and in pure stands under exceptional conditions, with a total area of about 30,000 ha. In this paper, the data of the studies carried out in the pedunculate oak forests of Bosnia and Herzegovina were compiled and a general evaluation was made. The results of phenological observation in pedunculate oak provenance test in Bosnia and Herzegovina through the years showed statistically significant differences among provenances in the beginning, end and duration of individual phenological phases. Researches on growth showed overtaking of provenances through the years, and best results in provenance Jelah for the most of investigated years. The research results on showed a significant positive correlation between the height and root collar diameter in provenance test and most of the investigated .The remaining pedunculate oak populations in Bosnia and Herzegovina have a good genetic structure and can be used as a seed stands for collecting seed material and producing seedlings for afforestation with this species.

Keywords: Pedunculate oak, Bosnia and Herzegovina, afforestation

Özet

Bosna Hersek Güneydoğu Avrupa'da yer almakta olup 51.129 km²'lik yüzölçümünün yarısından fazlası ormanlık alanlardan oluşmaktadır. Bosna-Hersek ormanlarının %93 ü doğal ormandır ve bir çoğu endemik olmak üzere çok sayıda bitki türüne sahiptir. Bosna Hersek'te sekiz meşe türü yetişmektedir. Bunlardan en önemlileri *Quercus robur* (XIX. yüzyılın sonlarında ve XX. yüzyılın başlarında aşırı kullanım nedeniyle neredeyse yok olmasına rağmen) ve *Quercus petraea*'dır. Bosna Hersek'teki saplı meşe ormanları, saf meşcereler, adi gürgenlerle karışık ve farklı koşullardan oluşan meşcereler halinde olmak üzere yaklaşık 30.000 hektarlık toplam alana sahiptir. Bu makalede, Bosna-Hersek'teki saplı meşe ormanlarında yürütülen çalışmaların verileri derlenerek genel bir değerlendirme yapılmıştır. Bosna-Hersek'te yapılan saplı meşe köken testinde yıllar boyunca süren fenolojik gözlemlerin sonuçlarına göre, bireysel fenolojik aşamaların başlangıcı, bitişi ve süresi açısından soy kökenleri arasında istatistiksel olarak anlamlı farklılıklar olduğunu göstermektedir. Büyüme üzerine yapılan araştırmalar, yıllar içinde kökenler arasında farklılıkların olduğunu göstermekte olup, çoğu incelenen yıl için en iyi sonuçlar Jelah kökeninde elde edilmiştir. Araştırma sonuçlarına göre, test edilen kökensel denemelerde boy ve kök boğazı çapı arasında anlamlı ve pozitif bir ilişkinin olduğunu belirlenmiştir. Araştırma sonuçları, Bosna Hersek'te kalan saplı meşe popülasyonlarının, tohum materyali toplamak, fidan üretmek ve ağaçlandırmalarda kullanmak için tohum meşceresi olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Saplı meşe, Bosna Hersek, ağaçlandırma

1. Introduction

Pedunculate oak (*Quercus robur* L.), and sessile oak (*Quercus petraea* (Matt.) Liebl.), are large, rugged, deciduous broadleaved trees, native to the majority of Europe. Both oaks reach northwards to southern Norway and Sweden, and southwards to the northern part of the Iberian Peninsula, South Italy, the Balkan Peninsula and Turkey. *Q. robur* has a more extended distribution, reaching more northerly ranges on the Norwegian coast and in northern Scotland; in Mediterranean areas it is also present in Portugal, Greece and South Turkey, and eastwards into continental central Russia, up to the Urals. The southerly range limits are difficult to define, as these oaks can mix, compete, and naturally hybridise with other Mediterranean oaks, such as *Quercus pubescen*s and *Quercus frainetto*. Both oaks occur at higher elevations in southern regions: *Q. robur* is recorded to grow up to 1300 m in the Alps, while *Q. petraea* is more montane and in southern Turkey can reach over 2,000 m. Due to the substantial human interest and usage of the species over many centuries, there has been widespread disturbance in their distribution, and the structure of their original forests is highly uncertain (Eaton et al., 2016).

Pedunculate oak represented an economically important species of Bosnian and Herzegovinian forests in the past (Memišević, 2008). At the beginning of the 20th century, Beck (1907) stated that there were only remnants of once large forest complexes of this species in Bosnia and Herzegovina. Begović (1960, 1978) confirmed this state in his research on the historical forestry development in Bosnia and Herzegovina.

Pedunculate oak in Bosnia and Herzegovina can be found in mixed forests with hornbeam, and under special conditions, it also builds pure stands of a permanent stage (Stefanović, 1977; Stefanović et al., 1983; Stefanović, 1986). The territory of Bosnia and Herzegovina represents the southeastern part of pedunculate oak natural distribution, and the species has a different genetic structure (Ballian et al., 2010a) compared with the central and northern parts of the range. Thus, pedunculate oak from Bosnia and Herzegovina, builds a specific connection between the southern and eastern provenances of the Balkan Peninsula and provenances from Central Europe across the Pannonian basin. Pedunculate oak from Bosnia and Herzegovina plays a significant role in the migration of genes from south to north and vice versa, and west to east and vice versa, which has been confirmed in several studies on glacial refuges (Petit et al., 2002; Slade et al., 2008).

According to the data from the first State forests inventory in 1964-1968 (Matić et al., 1971), the area of other high forests was 32,368 ha, of which, 31.7% or 10,261 ha were pedunculate oak forests. Unlike Matić et al. (1971), Klepac (1988) stated that the total area of pedunculate oak forests in Bosnia and Herzegovina was about 30,000 ha, and the best quality pedunculate oak forests were located in the municipalities of Bosanski Šamac, Bosanska Gradiška, Brčko and Bijeljina. The total area of these quality forests was about 14,000 ha. The oldest pedunculate oak forests were located in the municipality of Bosanska Gradiška, and average wood stock was 315 m3/ha. The rest were private, mostly coppice oak forests throughout Bosnia and Herzegovina.

Beck (1907) was the first to write in more detail about pedunculate oak in Bosnia and Herzegovina. He stated that pedunculate oak formed the Slavonian oak forest in the Sava plain, around the major tributaries of the Sava River, and also inside of Bosnia and Herzegovina, but that these were only the remains of once large forest complexes. Pedunculate oak can also be found in the valleys of larger rivers, such as Drina, Bosna, Lepenica, Lašva, Usora, Ukrina, Vrbanja, Vrbas, Unac, Una and Sana, in some of the karst fields, and on high mountain plateaus such as Glasinac (Jovančević, 1966, 1968), Petrovačko polje and Čađavica (Memišević, 2008). Management of these forests is also complicated by their ownership structure because they are mostly privately owned, not managed adequately, and are in an advanced stage of degradation.

This research aims to systematize the results of all previous research on pedunculate oak in Bosnia and Herzegovina, including research on leaf morphology in natural populations, growth, tree quality, phenology in the provenance test, as well as molecular research in natural populations and provenance test. Some suggestions were made regarding planning the measures of forestry works in the natural populations of pedunculate oak in Bosnia and Herzegovina, as well as the selection of the best provenances in terms of productivity and quality of wood mass and resistance to late frosts.

2. Research of morphological traits of leaves of pedunculate oak in B&H

The research of the leaf morphological traits of pedunculate oak assumed that indicators of variability at the intra-population and inter-population levels will be obtained. Variability for the analyzed properties would indicate the possibility of its use when performing forestry works in natural populations, as well as in the selection of seed stands,

groups of trees, and the best individuals for collecting seed and producing reproductive material.

In the morphological research of pedunculate oak leaves, Bašić et al. (2007) investigated four natural populations from northern Bosnia. Ballian et al. (2010b), Memišević (2010), Ballian et al. (2015), Ballian and Memišević Hodžić (2016), Ballian et al. (2017) investigated 44 natural populations of pedunculate oak in Bosnia and Herzegovina (figure 1). A total of 14 properties were analyzed.

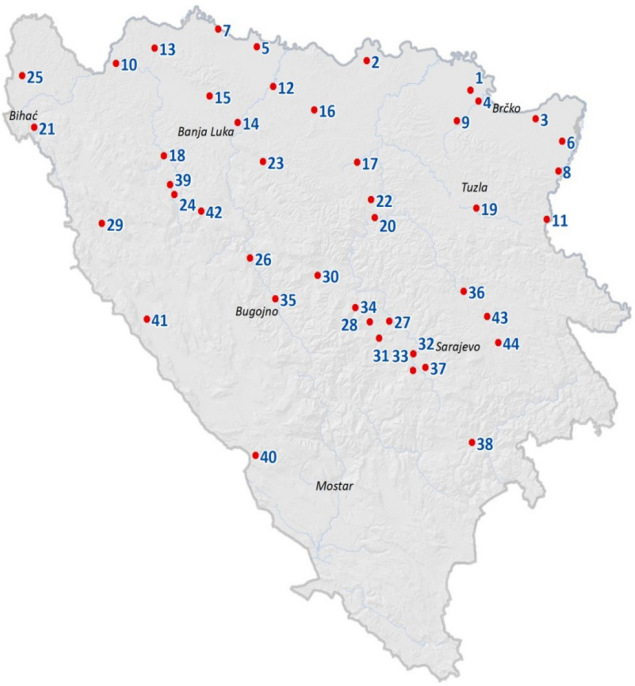
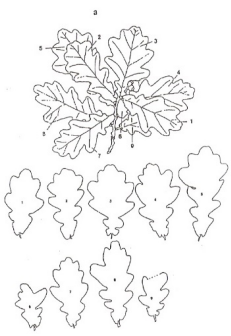
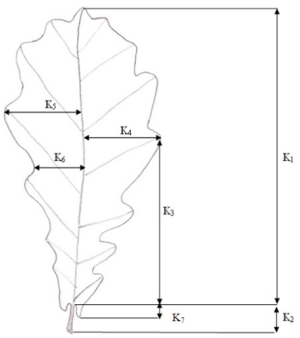


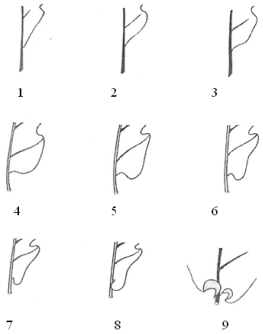
Figure 1. The research area of pedunculate oak in Bosnia and Herzegovina
Şekil 1. Bosna-Hersek'te saplı meşe araştırma alanı



Shoot with leaves, appropriate for leaf morphology research (Trinajstić, 1989; Franjić, 1996a,b,c)



Measured traits (Trinajstić, 1989; Franjić, 1996a,b,c)



Leaf base shape (Kremer et al., 2002)

Figure 2. Selection of leaves for analysis and measured and estimated traits
Şekil 2. Analiz için yaprakların seçimi, ölçülen ve tahmin edilen özellikleri

The results of research on morphological variability of leaves in natural populations of pedunculate oak in Bosnia and Herzegovina showed statistically significant variability among and within populations (Bašić et al., 2007; Ballian et al., 2010b; Memišević, 2010; Ballian et al., 2015; Ballian and Memišević Hodžić, 2016; Ballian et al., 2017).

The results of the research on the pedunculate oak leaves morphological variability showed statisti-

cally significant differences among populations from different altitudes (Ballian et al., 2015; Ballian et al., 2017).

3. Researches on phenology of pedunculate oak

The research was carried out in the Bosnian-Herzegovinian provenance test of pedunculate oak, established in the Žepče-Lugovo nursery in the spring of 2009. The experiment includes 28 provenances originating from Bosnia and Herzegovina (Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016; Memišević Hodžić and Ballian, 2018; Memišević Hodžić and Ballian, 2020a).

Field observations of leafing phenology were carried out in the spring of 2012, 2013, 2016, and 2019. Phenological monitoring was carried out from the end of March to the beginning of May. During that period, six different phenophases were monitored, according to the methodology given by Derory (2006) for sessile oak: Stage 0, bud is quiescent and protected by scales; Stage I, swelling bud; Stage II, opening of the bud has occurred; Stage III, leaves have grown; Stage IV, one leaf at least is completely out of the bud; Stage V, internodes have started growing. Collected data obtained by phenological observations of plants were statistically processed using the IBM SPSS Statistics 20 and Microsoft Excel 2007 packages.

The results showed statistically significant differences among provenances in the beginning, end and duration of individual phenological phases. The research results showed that provenance Bijeljina had a high rate of intra-provenance phenological variability and the earliest occurence of phenophases, and provenance B. Dubica the latest occurence into phases (Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016; Memišević Hodžić and Ballian, 2018).

Memišević Hodžić and Ballian (2020a) also researched the influence of season climate on phenological movements of pedunculate oak in the provenance test in 2016 and 2019. As in previous researches, the results showed that Bijeljina provenance entered the last stage (fully open leaves) at the earliest and Bosanska Dubica at the latest. Multivariate analysis of variance showed that the duration of phenophases depends on the year, provenance and interaction of provenances-years.

4. Researches on survival, growth and wood quality in provenance test

Research on survival, growth dynamics, and wood quality of pedunculate oak provenances were carried out in the Bosnian-Herzegovinian provenance test (Table 1).

At the end of the vegetation period in 2009, the first count and measuring of plants was carried out to determine the survival of young plants (Ballian et al., 2011). Survival results (Ballian et al., 2011) showed that provenances respond differently to planting and physiological stress. The average survival by provenances ranged from 61.10% for Bugojno to 89.81% for Kiseljak. The average survival rate for the provenance test was 75.02%.

The heights and root collar diameters were measured in the spring of 2012 (4-year-old plants), 2013 (5-year-old plants), and 2014 (6-year-old plants) (Memišević Hodžić, 2015, Ballian and Memišević Hodžić, 2016).

The variance analysis for one-year-old plants for all investigated traits showed statistically significant differences among the investigated provenances. The Jelah provenance had the highest average value of height, 47.41 cm, and the lowest average height Mutnica provenance, 25.76 cm (Ballian et al., 2011).

In the research of Memišević Hodžić (2015) and Ballian and Memišević Hodžić (2016) for the height, variance analysis showed statistically significant differences among provenances. The lowest average height in the spring of 2012 was in provenance Miljevina, 2013 in Stojčevac, and 2014 in Stojčevac. The highest average height in 2012, 2013 and 2014 was in provenance Jelah.

For the root collar diameter, variance analysis also showed statistically significant differences among provenances. In the spring of 2012, the Vinac provenance had the smallest average root collar, and in 2013 and 2014 Stojčevac provenance. Jelah provenance had the highest average root collar diameter in 2012, 2013, and 2014 (Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016).

Memišević Hodžić and Ballian (2019) researched growth tendency of pedunculate oak provenances in provenance test in Bosnia and Herzegovina in relation to fixation index, and found that eight out of twenty provenances showed positive fixation index mean values. Those provenances were: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad and Sokolac (Miljevina Foča, Bosanska Dubica, and Drvar provenances with the highest positive values of fixation index). Olovo provenance had the lowest negative fixation index mean value, -0.1269.

Memišević Hodžić and Ballian (2020b) researched branching angle and stem form on provenances in pedunculate oak provenance test in Bosnia and

Table 1. List of provenances in the provenance test
Tablo 1. Orjin denemesinde kullanılan orjinlerin listesi

No	Provenances	Locality	Latitude	Longitude	Altitude	Isosyme analysis
1	Bijeljina	Patkovača	44º 43’ 50’’	19º 13’ 30’’	93	+
2	Bosanska Dubica	Knežica	45º 06’ 24’’	16º 40’ 32’’	145	+
3	Bosanska Gradiška	Lipnica	45º 06’ 64’’	17º 18’ 63’’	91	+
4	Bosanski Brod	Zborišta	45º 05’ 27’’	18º 00’ 38’’	84	+
5	Bosansko Grahovo	Crni lug	44º 01’ 05’’	16º 38’ 24’’	703	+
6	Bugojno	Kopčić	44º 06’ 00’’	17º 26’ 31’’	537	+
7	Drvar	Unac	44º 23’ 39’’	16º 21’ 54’’	462	+
8	Hrgovi Srebrenik	Dubrava	44º 49’ 06’’	18º 34’ 11’’	133	+
9	Jelah	-	44º 39’ 09’’	17º 56’ 46’’	181	+
10	Kaćuni	Nezirovići	44º 03’ 59’’	17º 56’ 13’’	443	+
11	Kiseljak	Dalmacija	43º 56’ 30’’	18º 04’ 56’’	477	-
12	Ključ	Velečevo	44º 30’ 56’’	16º 48’ 42’’	260	-
13	Knežina	-	44º 01’ 40’’	18º 44’ 53’’	759	+
14	Kotor Varoš	-	44º 39’ 07’’	17º 21’ 35’’	252	-
15	Lukavica	-	43º 49’ 26’’	18º 21’ 58’’	552	+
16	Miljevina Foča	Miljevina	43º 31’ 06’’	18º 38’ 56’’	627	+
17	Mrkonjić Grad	Čađavica	44º 27’ 04’’	16º 58’ 42’’	753	+
18	Mutnica Cazin	-	44º 58’ 55’’	15º 50’ 54’’	270	+
19	Nević polje	-	44º 11’ 46’’	17º 42’ 11’’	476	-
20	Novi Šeher	-	44º 30’ 09’’	18º 02’ 02’’	230	-
21	Olovo	Olovske Luke	44º 07’ 44’’	18º 36’ 11’’	542	+
22	Sokolac	Lug	43º 55’ 17’’	18º 48’ 53’’	866	-
23	Stojčevac Ilidža	-	43º 48’ 40’’	18º 17’ 25’’	506	-
24	Vinac	Bila Voda	44º 15’ 48’’	17º 17’ 08’’	408	-
25	Visoko	Muhašinovići	44º 00’ 38’’	18º 08’ 45’’	413	-
26	Zavidovići	Grad	44º 26’ 07’’	18º 07’ 49’’	210	-
27	Žepče	Žepački lug	44º 25’ 35’’	18º 03’ 10’’	224	+
28	Živinice	D. Dubrave	44º 27’ 58’’	18º 41’ 09’’	216	+

Herzegovina. Variance analyses showed statistically significant differences among provenances. Plants with branching angle <22.5° were found in 2% of the total number of plants, branching angle 22.5°–45° in 19% of plants, branching angle 45°–67.5° in 32% of plants, and branching angle 67.5°–90° in 47% of plants. The highest percentage of the most favorable branching angle had a provenance from Ključ, Miljevina Foča, and Bugojno. Category 10 of stem form (ideal tree, without defect) was found in 14% of the plants, category 9 (a small defect) in 16%, category 8 (two slight defects) in 3%, category 7 (two medium defects or many small defects) in 4%, category 6 (significant defects that can be recovered or more medium defects) in 16%, and category 5 (many defects) in 7% of the total number of plants. In category 4, the researchers recorded all the plants without silvicultural value, including 40%. The highest percentage of category 10 plants was in Drvar provenance (42%), whereas the lowest was in Bugojno provenance (2%).

5. Research on correlation between natural populations and their offspring

Ballian and Memišević Hodžić (2022) researched height and root collar diameter traits in provenance test of pedunculate oak, and correlations between their growth and leaf morphological traits in populations of their origin.

Statistically significant differences were found among the investigated provenances for height and root collar diameter. The highest average plant height was for Drvar provenance (445.8 cm), followed by Jelah (436.0 cm) and Vinac (432.0 cm). Visoko-Muhašinovići provenance had the lowest average plant height (262.3 cm). In 2020, an overtaking of provenances was recorded for the plant height. Živinice provenance had the highest average root collar diameter (10.7 cm), followed by the Jelah and Drvar provenances. Nević polje provenance had the lowest average root collar diameter (7.4 cm).

A significant positive correlation was recorded between the height and root collar diameter, as well as between the growth trait (height and root collar diameter) and most of the investigated morphological traits of the leaves in the populations of their origin.

6. Molecular research (DNA analysis)

The research on the genetic variability of pedunculate oak in Bosnia and Herzegovina using SSR microsatellites was carried out by Ballian et al. (2010a). Fourteen populations from Bosnia and Herzegovina were analysed, with 20 trees per population (except Kiseljak with 10 trees, and Novi Šeher with 5 trees). Four SSR markers were used (ssrQpZAG1/5, ssrQpZAG9, ssrQpZAG36, ssrQpZAG108). DNA was extracted from vegetative material by means of SIGMA „GenEluteTM Plant GenomicDNAMiniprep Kit". In order to produce the polymerase chain reaction (PCR), a slightly modified procedure according to Lefort et al. (1998) and Wilhelm et al. (2005) were used

Table 2. Researched populations of pedunculate oak (Ballian et al., 2010a)
Tablo 2. Araştırılan saplı meşe populasyonları

No	Population	Latitude	Longitude	Altitude (m)	Number of individuals
1.	Cazin	44°58'06''	15°56'04''	352	20
2.	Bosanska Gradiška	45°08'29''	17°11'38''	85	20
3.	Bosanski Brod (Zborište)	45°03'01''	18°00'04''	91	20
4.	Orašje (Obudovac)	45°01'26''	18°33'37''	80	20
5.	Mrkonjić grad (Podbrdo)	44°26'21''	16°59'52''	727	20
6.	Jelah	44°39'11''	17°57'23''	185	20
7.	Žepče	44°25'46''	18°03'21''	219	20
8.	Živinice (Dubrave)	44°26'46''	18°40'26''	226	20
9.	Livno (Crni lug)	44°00'53''	16°37'48''	697	20
10.	Bugojno (Kopčić)	44°05'22''	17°26'08''	539	20
11.	Sarajevo (Stojčevac)	43°48'55''	18°16'54''	494	20
12.	Romanija (Sokolac)	43°55'51''	18°48'06''	847	20
13.	Novi Šeher	44°30'51''	18°01'22''	242	5
14.	Kiseljak	43°56'26''	18°04'55''	473	10

The successfully sequenced amplified samples from the analyzed populations and statistical data processing resulted in 108 alleles. The average number of alleles per population was 11.83. The number of alleles in the Žepče population was 10.00, and in Cazin 14.25, which can be attributed to stronger anthropogenic influences on Žepče population through history. Observed heterozygosity was smaller than expected heterozygosity in all the cases. The highest observed heterozygosity was in provenance Cazin (0.838) and the lowest Bugojno and Mrkonjić Grad (0.563). The fixation index showed positive values. Its value in the Cazin population was very low, 0.074. The highest values were found in small and isolated Bosanski Brod, Livno, Bugojno, and Mrkonjić Grad populations. The average value of the fixation index was positive, 0.234. Fixation index analyzed per loci also showed positive values. The Bosnian-Herzegovinian populations show relatively high intra-population diversity, with the average for all loci Fis = 0.273, and low inter-population differentiation in all the investigated loci, with Fst = 0.051. The analysis of genetic distances showed that, according to Nei (1972), the highest genetic distance was between Orašje and Romanija populations, and the lowest between Bosanska Gradiška and Živinice.

7. Biochemical research

Memišević Hodžić (2015) conducted an isozyme analysis in the Bosnian-Herzegovinian provenance test of pedunculate oak in Žepče. Twenty provenances were investigated, with 50 examined individuals per provenance (table 1). Enzymes were extracted from dormant buds and separated using starch-gel electrophoresis. Ten isoenzyme systems were used (E.C. numbers and controlled loci given in parentheses): alanine aminopeptidase (E.C. 3.4.11.1.; Aap-A), aspartate aminotransferase (E.C. 2.6.1.1., Aat-B), fluorescent a-esterase (E.C. 3.1.1.1.; Fest-A, Fest-C), alcohol dehydrogenase (E.C. 1.1.1.1; Adh-A), isocitrate dehydrogenase (E.C.

1.1.1.42.; Idh-A, Idh-B, Idh-C), menadione reductase (E.C. 1.6.1.99.2.; Mnr-A), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44.; 6-pgdh-A, 6-pgdh-B), phosphoglucose isomerase (E.C. 5.3.1.9.; Pgi-B), phosphoglucomutase (E.C. 2.7.5.1.; Pgm-B), and shikimate dehydrogenase (E.C. 1.1.1.25.; Skdh-A). Electrophoretic and staining procedures followed Konnert et al. (2004).

The results showed that nine of the 14 analyzed loci were polymorphic, while the rest were relatively monomorphic. A high degree of polymorphism was found at gene loci AAP-A, PGM-B, IDH-A, IDH-B, and IDH-C. Provenances Bosanska Dubica, Bosansko Grahovo, Kotor Varoš, Olovo, and Stojčevac had the lowest mean value of the number of alleles per locus (2.36), and Bosanski Brod the highest (3.14). Provenances Drvar and Miljevina had high mean values of the number of alleles per locus (3.07). The provenances of Bosanska Dubica, Mutnica, and Stojčevac had the lowest percentage of polymorphic loci (50.00%), and Živinice the highest (85.71%). The average number of genotypes per provenance ranged from 2.79 in the Olovo provenance to 4.00 in the Drvar provenance. The highest observed heterozygosity had Olovo provenance with 0.2907, and the lowest Bosanska Dubica with 0.1571. The observed heterozygosity was higher than expected for eight provenances: Bosanski Brod, Hrgovi Srebrenik, Jelah, Kaćuni, Kotor Varoš, Olovo, Stojčevac, and Žepče. The highest expected mean heterozygosity for all gene loci had Jelah provenance, 0.2804, and the lowest Stojčevac, 0.1867. The average value of the fixation index for all populations in research of Memišević Hodžić (2015) was positive, 0.0033. Eight of the 20 provenances showed positive mean values of the fixation index: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad, and Sokolac. The lowest negative value of the fixation index had the provenance Olovo with -0.1269. The Stojčevac provenance had the lowest values of multilocus and gene pool diversity and intra-provenance differentiation, and the Jelah provenance had the highest. The lowest multilocus deviation was between provenances Bijeljina and Mrkonjić Grad, and the largest between Olovo and Kotor Varoš. The lowest mutual deviation of the gene pool had provenances Bosanski Brod and Stojčevac, and the highest Olovo and Žepče.

Memišević et al. (2021) investigated the genetic variability of pedunculate oak at the Mediterranean margin of the distribution range. To assess the patterns of gene diversity distribution at the rear edge of the Holocene colonization, they studied genetic variation in 20 pedunculate oak populations using 14 allozyme loci (as previously mentioned for isosyme analysis). Despite considerable differences among populations, neither the numbers of alleles nor genetic diversity showed any geographical trend within the studied area, although small, isolated populations showed generally lower allelic richness. The Bayesian analysis of population structure indicated a kind of geographical pattern. Tests of a recent bottleneck have not identified a heterozygosity excess in any population. The proximity to multiple glacial refugia explains the outcomes.

Except for the population Bosanska Dubica, all large and medium populations were at Hardy-Weinberg equilibrium, and the fixation indices were near zero.

8. Discussion And Conclusion

In this article, the results of research on leaf morphology and molecular research in natural populations, as well as growth, tree quality, phenology, and molecular research in the provenance test, along with the correlation of some traits in natural populations and provenance test, were presented.

The results of research on morphological variability of leaves in natural populations of pedunculate oak in Bosnia and Herzegovina showed statistically significant variability among and within populations (Bašić et al., 2007; Ballian et al., 2010b; Memišević, 2010; Ballian et al., 2015; Ballian and Memišević Hodžić, 2016; Ballian et al., 2017). Similar results were obtained in Croatia by Franjić (1993, 1994a, b, 1996a,b,c). Enescu et al. (2010) found significant differences in petiole ratio, petiole length and lobe depth ratio, highly significant differences in basal shape, and non-significant differences in other traits among seven pedunculate oak stands in Romania.

Research on the spring phenology of pedunculate oak is necessary in areas where late frosts occur. The pronounced intra-population variability of the beginning of leafing of pedunculate oak was found in the last century by Cieslar (1923), Hesmer (1955), Krahl-Urban (1959), Šafar (1966) and Stojković (1991).

The results of research on the spring phenology of pedunculate oak in the provenance test showed statistically significant differences among provenances in the beginning, end and duration of individual phenological phases. As pedunculate oak is a species sensitive to late spring frosts, which often occur in Bosnia and Herzegovina, phenological research is important for the selection of suitable provenances during forestry work. The be-

ginning of vegetation depends on the high spring temperatures and precipitation, and it is necessary to perform multi-year observations. The research results showed that provenance Bijeljina, located at an altitude of 93 m near the Drina River - high rate of intra-provenance phenological variability and the earliest occurrence of phenophases, while provenance B. Dubica, also from a low altitude (145 m), had the latest occurrence into phenophases (Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016; Memišević Hodžić and Ballian, 2018). Expectations that provenances from higher altitudes (Sokolac, Knežina, B. Grahovo) will enter the phases later were not met. Considering that differences are only about 15 days, it is too early to "declare" an early or late form of species. In the research of Bobinac et al. (2012), the phase of fully developed leaves appears a little later than in the research of Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016; Memišević Hodžić and Ballian, 2018; and Memišević Hodžić and Ballian 2020a.

The results of research on the influence of season climate on the phenological movements of pedunculate oak in the provenance test in 2016 and 2019, as shown by Memišević Hodžić and Ballian (2020a), also indicated that the Bijeljina provenance entered the last phase (fully open leaves) at the earliest, while Bosanska Dubica entered it at the latest. Multivariate analysis of variance showed that the duration of phenophases depends on the year, provenance and interaction of provenances-years. The results confirmed the influence of heredity of leafing properties and the dependence of phenological phases on climatological conditions. Research needs to be continued to determine the extent to which daily and monthly temperatures and precipitation affect the beginning of the phenological phases of leafing.

As pedunculate oak is a species sensitive to late spring frosts, the results of observing the leafing phenology should be used in the selection of provenances for afforestation in a particular area. Since the authors (Memišević Hodžić 2015; Ballian i Memišević Hodžić 2016; Memišević Hodžić i Ballian 2018, Memišević Hodžić and Ballian 2020a) observed the leafing phenology in pedunculate oak provenance test only in four years, it is early to claim when the vegetation of pedunculate oak in the provenance test Žepče begins. Multi-year observations are needed because the beginning of the vegetation depends on the height of the spring temperatures, as well as the amount of precipitation. In depressions and similar forms of terrain, primarily due to low night temperatures, the tempo of plant development slows down or accelerates (Kramer, 2001).

Results of research on survival showed that the average survival rate for the provenance test was 75.02% (from 61.10% for Bugojno to 89.81% for Kiseljak). Results of the research on growth dynamics showed statistically significant differences and overtaking among provenances through different years, but provenances Jelah was the best in height and thickness increase in the first years (Ballian et al., 2011; Memišević Hodžić, 2015; Ballian and Memišević Hodžić, 2016). Provenance Drvar had the highest average height in 2020, and provenance Živinice had the highest average root collar diameter (Ballian and Memišević Hodžić, 2022). Other researchers from the region also found statistically significant differences among the investigated provenances (Gračan, 1995; Perić, 2001; Perić et al., 2000, 2003, 2006; Roth, 2003, 2006). The results of average heights of four-year-old plants obtained by Popović et al. (2014) were higher than those obtained by Memišević Hodžić (2015). On the contrary, the average heights and root collar diameters obtained by Memišević Hodžić (2015) for five-year-old plants were higher than those obtained by Roth (2006) in Croatia. The reasons for the differences between the two studies can be found in the applied agrotechnical measures during production in the nursery, as well as in the different ecological conditions.

In the research on the growth tendency of pedunculate oak provenances in relation to the fixation index, eight out of twenty provenances showed positive fixation index mean values: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad, and Sokolac (Memišević Hodžić and Ballian, 2019), which indicates the presence of inbreeding in the population of origin. Olovo provenance had the lowest negative fixation index mean value, -0.1269. The positive fixation index value of the Miljevina provenance matches its low growth, while this is not the case for the Drvar and Bosanska Dubica provenances. 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.

Variance analyses showed statistically significant differences in branching angle and stem form among provenances (Memišević Hodžić and Ballian, 2020b). The highest percentage of the most favorable branching angle had a provenance from Ključ, Miljevina Foča, and Bugojno. The highest percentage of category 10 plants was in Drvar provenance (42%), whereas the lowest was in Bugojno provenance (2%).

A significant positive correlation was recorded between the growth trait (height and root collar diameter) in the provenance test and most of the investigated morphological traits of the leaves in the populations of their origin. These results could be useful in pedunculate oak stands, where the offspring of individuals selected on the base of macromorphological parameters of leaves is expected to grow fast.

The research results on the genetic variability of pedunculate oak in Bosnia and Herzegovina using SSR microsatellites indicated the presence of high polymorphism in the analyzed microsatellites, i.e., genetic diversity even in the small, isolated populations included in this research (Ballian et al., 2010a). Observed heterozygosity was smaller than expected heterozygosity in all the cases. The existing differences between observed and expected heterozygosity show the real condition's deviation from the state of balance. This results might be expected due to the size of the population and the condition of all natural populations of pedunculate oak in Bosnia and Herzegovina. The fixation index showed positive values, which indicates the presence of inbreeding. Still, its value in the Cazin population was very low, 0.074. The reason could be the openness of the population and its relatively well preserved condition, as well as possible gene flow with Croatian populations from the Pokuplje area. The highest values were found in small and isolated Bosanski Brod, Livno, Bugojno, and Mrkonjić Grad populations. Since Bosanski Brod belongs to the Posavina area and is divided from the renowned Slavonian forests of pedunculate oak only by the River Sava, this is an unexpected value. This situation can largely be attributed to the reduced number of trees in the area of Bosanski Brod and to significant historical influences. In contrast to these populations, the Romanija population showed an unexpectedly small value, keeping in mind that it is relatively isolated, small, and devoid of any possibility of gene flow. The average value of the fixation index was positive at 0.234. The authors (Ballian et al., 2010a) considered this to be the consequence of considerable fragmentation of pedunculate oak. The Bosnian-Herzegovinian populations show relatively high intra-population diversity, with the average for all loci Fis = 0.273, and low inter-population differentiation in all the investigated loci, with Fst = 0.05.

The analysis of genetic distances showed that, according to Nei (1972), the highest genetic distance was between Orašje and Romanija populations, which are geographically distant, with many orographic barriers between them. The lowest genetic distance was found between Bosanska Gradiška and Živinice, which was expected since only Mount Majevica is between Živinice and Posavina lowland, where Bosanska Gradiška is situated. Nevertheless, it was expected that the Živinice population would be closer to the Orašje population. These results showed that the three populations of pedunculate oak from the area of Bosanska Posavina: Orašje, Bosanski Brod, and Bosanska Gradiška, deviate from one another. However, these populations were expected to show the smallest genetic distance. In terms of pairwise F_{ST} values, there is a minimal value between the populations of Bosanska Gradiška and Livno and a maximal value between the populations of Orašje and Bugojno. Genetic variability, based on microsatellite analyses, was reported by Steinkellner et al. (1997), Lefort et al. (1998) Barreneche et al. (1998), Lexer et al. (2000), Wilhelm et al. (2005). They analyzed different developmental stages of pedunculate oak and its embryos.

The results of isosyme analysis of pedunculate oak provenances in Bosnian-Herzegovinian provenance test showed that nine of the 14 analyzed loci were polymorphic, while the rest were relatively monomorphic. A high degree of polymorphism was found at gene loci AAP-A, PGM-B, IDH-A, IDH-B, and IDH-C. Provenances Bosanska Dubica, Bosansko Grahovo, Kotor Varoš, Olovo, and Stojčevac had the lowest mean value of the number of alleles per locus (2.36), and Bosanski Brod the highest (3.14). Provenances Drvar and Miljevina had high mean values of the number of alleles per locus (3.07). Yakovlev (2000) obtained lower values of the mean number of alleles per locus (1.80 to 2.50) in pedunculate oak populations from the central part of Russia, and Gömöry et al. (2001) for pedunculate oak populations from Bulgaria, Slovakia, and Russia, while Crăciunesc et al. (2011) obtained a higher number of alleles per gene locus for pedunculate oak from the Prejmer nature reserve. The highest observed heterozygosity had Olovo provenance with 0.2907, and the lowest Bosanska Dubica with 0.1571. These values are higher than those determined by Yakovlev (2000) in the study of pedunculate oak populations in the central part of Russia. In the research of Ballian et al. (2010a) with the use of SSR markers, all twelve analyzed populations had observed heterozygosity lower than expected. It was explained by the size of populations of pedunculate oak in Bosnia and Herzegovina and their conditions. The total mean value of observed heterozygosity in the research by Memišević Hodžić (2015) was slightly higher than that determined by Crăciunesc et al. (2011) for the twelve gene loci analyzed and which was

0.229. Gömöry et al. (2001) found the actual heterozygosity of pedunculate oak populations from Russia to be 0.161, populations from Slovakia to be 0.122, and from Bulgaria to be 0.179, which was explained by a consequence of the long migration of this species from the west to the east. The highest expected mean heterozygosity for all gene loci had Jelah provenance, 0.2804, and the lowest Stojčevac, 0.1867. The values obtained in the research of Memišević Hodžić (2015) were higher than those determined by Yakovlev (2000), which ranged from 0.119 to 0.184 for different populations, and those determined by Gömöry et al. (2001) for populations from Russia (0.163), from Slovakia (0.136), and from Bulgaria (0.166). The overall mean value of expected heterozygosity in the research of Memišević Hodžić (2015) was slightly lower than that determined by Crăciunesc et al. (2011).

The average value of the fixation index for all populations in the research conducted by Memišević Hodžić (2015) was positive, at 0.0033. Eight of the 20 provenances showed positive mean values of the fixation index: Bijeljina, Bosanska Dubica, Bosansko Grahovo, Drvar, Jelah, Miljevina, Mrkonjić Grad, and Sokolac. The lowest negative value of the fixation index had the provenance Olovo with -0.1269. In the research conducted in Bosnia and Herzegovina by Ballian et al. (2010a), the fixation index had positive values for all populations. Crăciunesc et al. (2011), in the Prejmer reserve in Romania, obtained the average fixation index for all gene loci, 0.052. In the research conducted by Yakovlev (2000), the average value of the fixation index for all tested gene loci was -0.001.

The results of research on the genetic variability of pedunculate oak at the Mediterranean margin of the distribution range showed that, except for the population Bosanska Dubica, all large and medium populations were at Hardy-Weinberg equilibrium, and the fixation indexes were near zero. In general, oaks (including pedunculate oak) are at HWE or exhibit slight heterozygote deficiency, whatever markers are used (Jensen et al., 2003; Belletti et al., 2005; Di Pietro et al., 2021). High differentiation typically accompanies fragmentation; this was also confirmed in Bosnia and Herzegovina. Despite the small territory under study, the overall differentiation coefficient was relatively high; however, this is quite typical for marginal oak populations (Vakkari et al., 2020; Degen et al., 2021; Di Pietro et al., 2021). Typically, interpopulation variation among isolated populations is attributed to genetic drift associated with reduced population sizes, i.e., the bottleneck effect in older fragments, and the founder effect in newly established populations.

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