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Original scientific article

GENETIC STRUCTURE OF SESSIL OAK (*Quercus petraea* (Matt.) Liebl) FROM THE AREA OF OUTSTANDING NATURAL BEAUTY "AVALA"

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The paper presents the results of the genetic diversity analysis of Sessile oak populations from the area of outstanding natural beauty (AONB) "Avala" which were obtained using SSR markers. Genomic DNA was isolated from leaf tissue of 50 test trees from two populations. Genotyping was performed using microsatellite markers QpZAG110, QpZAG15, QpZAG1/2, QpZAG3/64, QpZAG36, QpZAG1/5, and QrZAG108. All loci were polymorphic with the high mean value of PIC (0.934). The total number of alleles determined in the studied population was 127. The range of alleles varies from 15 (QpZAG1/5, QpZAG1/2) to 23 (QpZAG110) with an average of 18.14 alleles per locus. The number of effective alleles ranges from 8.273 (QpZAG1/5) to 13.830 (QrZAG108). The mean value of the gene flow (Nm) was 8.522 with a range from 5.548 to 14.876. Overall genetic diversity was high (He = 0.909) and ranged from 0.879 to 0.928. Due to the excess of homozygotes observed at most loci, a significant inbreeding coefficient was detected (Fis = 0.796). The Analysis of Molecular Variance (AMOVA) confirmed that genetic diversity was more pronounced within populations (77.5%) than between them (1.6%). The average allele frequency (Q) of the studied populations shows that the individuals originate from two or more populations. The obtained results can be used for the adoption of appropriate plans for the management of protected natural resources and the management of this ecologically and economically important tree species. Also, the

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obtained results enable the adoption of the necessary measures for the conservation of sessile oak genetic resources by in-situ and ex-situ methods. Based on the research results, the use of this important species can be recommended for its reintroduction in optimal microclimatic conditions, as well as in the selection of the best individuals for the reintroduction.

Key words: differentiation, population, SSR (Simple Sequence Repeat) markers

INTRODUCTION

Oaks (*Quercus* sp.) are the most represented tree species in the forests of Serbia, after beech. In addition to its distribution by species number, oaks are one of the most important systematic groups in the dendroflora of Serbia. Sessile oak (*Quercus petraea* (Matt.) Liebl.) participates in the growing stock of the Republic of Serbia with 6.1%, according to number of trees. Its share in total volume is 5.9% while it participates in volume increase with 6.1% (NFI, 2008). New trends in the number of trees will be possible to evaluate after the completion of a new forest inventory, which is ongoing. Sessile oak is included in the in-situ conservation programs in Serbia through 13 seed stands for production of selected reproductive material with a total area of 82.81 ha. There are also 32 seed objects for production of reproductive material of known origin, with a total area of 775.82 ha (DOF, 2020).

The species mainly occurs on warmer, southern exposures within the alliance of *Quercion petraeae-cerris* Lakš. et Jov. 1980. What is evident is that negative activities in the recent and distant past have caused major changes in natural ecosystems, which have gradually led to decline of populations, and their habitats have been destroyed or reduced (BOROVICS and MÁTYÁS, 2013; TORRES-RUIZ *et al*., 2019; ŠIJAČIĆ-NIKOLIĆ *et al*., 2020).

Due to the great economic importance of the sessile oak and its populations, as well as the high degree of vulnerability due to the effects of drought, numerous studies of its ecological variability in Serbia were conducted (CVJETIĆANIN *et al*., 2005; KRSTIĆ *et al*., 2017). MILOVANOVIĆ (2009) identified eight gene-ecological zones based on the climatic, edaphic and phytocenological characteristics of the localities where sessile oak forests have been recorded in Serbia. These gene-ecological zones were the starting point for the collection of plant material for laboratory analyses at the level of molecular markers.

The genetic variability of sessile oak has been investigated across Europe using various molecular markers (KREMER and PETIT, 1993; DUMOLIN *et al*., 1997; PETIT *et al*., 1997; FERRIS *et al*., 1998; LE CORRE *et al*., 1997; BORDÁCS *et al*., 2002; FINESCHI, 2002; PETIT *et al*., 2002, 2004; PORTH *et al*., 2005; BALLIAN *et al*., 2007; UENO *et al*., 2011; BATOS *et al*., 2017, etc.).

The genetic variability of sessile oak in Serbia has been determined using universal primer pairs of chloroplast DNA. Five different sessile oak haplotypes were detected (ŠIJAČIĆ-NIKOLIĆ *et al*., 2009; 2009a), which served as a basis for defining of two provenance regions ("Official Gazette of the Republic of Serbia" No. 91/08). The results of molecular analyses showed that the regions of the gene-ecological zones of Vojvodina, Northwestern Serbia, Sumadija, Northeastern, Eastern, Southeastern, and Central Serbia can be combined into one region (Region I), since the presence of only one haplotype in all sampled populations from

these zones. The gene-ecological zone of Western and Southwestern Serbia, including the entire range of altitudes in which sessile oak populations are present, is defined as a separate region (Region II), since rare haplotypes are present. This conclusion is confirmed by the ecological characteristics of the sessile oak sites in this region, which are characterized by the specific geological basis (serpentinite, serpentinized peridotite, and peridotite), as well as three different types of climate (humid low forests climate, humid high forests climate, and moist perhumid climate).

Sessile oak trees in forest ecosystem of the area of outstanding natural beauty (AONB) "Avala", near the capital of Serbia – Belgrade, are exposed to a higher level of human threats, due to its specific location and environment (ALVEY, 2006; FAO, 2016). The AOBN "Avala" covers an area of 855 ha, of which 761 ha are overgrown with forest. The total volume is 144.024 m3 or 168.8 m3/ha. The average number of trees per hectare is 392. Sessile oak is the most common species, out of a total of 37 species present in the area. The total number of sessile oak trees is 9.646 with a total volume of 7.268 m3. An artificially raised sessile oak seed stand on the area of 0.50 hectares was singled out in the AONB "Avala". Due to the deteriorating condition of coppice sessile forests, there is a growing need for regeneration, and thus for the production of seeds and seedlings. There are frequent weather disasters, such as the stormy wind that hit the Avala mountain top in 2016 on the area of 200 ha (SRBIJAŠUME, 2017).

The forest areas near the city also possess an added value as refuge habitats of species, important areas for preservation of nature integrity, as well as for improving the quality of urban life. Accordingly, it is necessary to investigate the genetic variability of the population in order to define adequate conservation measures, which will improve all ecosystem functions.

POPOVIĆ *et al*. (2020) investigated intra-population variability of sessile oak in AOBN "Avala" using 11 leaf morphological traits as adaptive markers. The results showed high intra-population variability for all observed traits. On the other hand, according to the previously mentioned results of chloroplast DNA variability, the Avala site is classified in the first region of provenance, which is characterized by the presence of only one haplotype.

The results of previous research have opened the possibility and laid the groundwork for the application of other molecular markers, which would indicate the degree of intra-population variability of sessile oak in AOBN "Avala".

The SSR (Simple Sequence Repeats or microsatellites) consists of DNA segments that contain numerous repeats of short, "motif" sequences, usually from one to six bases. Microsatellites are suitable for studying gene fluctuation, effective population size, migration and dispersion processes, parenting and degree of relatedness (GODOY and JORDANO, 2001). Previous research has shown a high degree of informativeness of SSR markers in the population and ecological research of different Quercus species (BARRENECHE *et al*., 2004; XU *et al*., 2004; UENO and TSUMURA, 2008; DURAND *et al*., 2010; FILIZ *et al*., 2012; LIND and GAILING, 2013; GUO, 2018; GUO *et al*., 2021; YÜCEDAĞ *et al*., 2021 and others).

The estimation of sessile oak population's variability in Serbia using SSR markers has not been done so far. The aim of this research is to perform molecular genetic identification of sessile oak from the area of AOBN "Avala" using SSR markers and based on the obtained results to provide guidelines for preservation of the available gene pool and prescribe measures for management of these forests.

MATERIAL AND METHOD

Plant material

For the needs of determination of sessile oak genetic diversity (*Quercus petraea* (Matt.) Liebl) in the AONB "Avala", the two populations had been distinguished, one on the south and the other on the east side of the mountain (Table 1). In July 2019, twenty-five test trees per population (a total of 50 test trees) were selected by a method of phenotypically superior genotypes selection, from which the samples for genetic analysis (fully developed, undamaged leaves) were taken.

Table 1. Geographic characteristics of analysed populations

Population Code	Aspect	Latitude	Longitude	Elevation (m)
I	S	44°41'11"	20°30'51"	410
II	N	44°41'52"	20°30'55"	420

DNA extraction and PCR amplification

A total genomic DNA was isolated from herbarised leaves using modified DOYLE and DOYLE (1987) protocol. The isolated DNA samples were quantified on Eppendorf BioSpectrometer® kinetic.

Table 2. List of seven informative primers, with their sequences, repeat motif, references and allele range

Locus	Primer Sequences	Repeat motif	Reference	Allele size range (bp)
QpZAG110	F:GGAGGCTTCCTTCAACCTACT R:GATCTCTTGTGTGCTGTATTT	(AG)15		184-248
QpZAG15	F:CGATTTGATAATGACACTATGG R:CATCGACTCATTGTTAAGCAC	(AG)23		101-144
QpZAG1/2	F:TCCTCCGCTCACTCACCATT R:AAACCTCCACCAAAACATTC	(AG)16	Steinkellner *et al.* 1997	97-122
QpZAG3/64	F:TAGAAAGCCCAAAACCAAAACC R:CTTTTTGGAAGCCGCTTCCGTA	(AG)21		151-205
QpZAG36	F:GATCAAAATTTGGAATATTAAGAGAG R:ACTGTGGTGGTGAGTCTAACATGTAG	(AG)19		194-229
QpZAG1/5	F:GCTTGAGAGTTGAGATTTGT R:GCAACACCCTTTAACTACCA	(GT)5(GA)9		161-202
QrZAG108	F:CTAGCCACAATTCAGGAACAG R:CCTCTTTTGTGAATGACCAAG	(AG)13	Kampfer *et al.* 1998	232-283

The Simple Sequence Repeat (SSR) characterization was done with seven polymorphic nuclear markers, previously developed for *Quercus petraea* and *Quercus robur* (Table 2).

Polymerase chain reaction (PCR) was carried out in 25 μL reaction volume containingfinal concentrations of next components: 25 ng of DNA sample, 1 x Buffer (Fermentas), 0.8 mM dNTP, 0.5 μM of each primer pair and 1U Taq polymerase (Fermentas). The PCRs were performed on thermocycler BiometraTProfessional Standard 96 using the following program: an initial denaturation at 94ºC/15min by 32 cycles each of denaturation at 94ºC/45s, annealing at different temperatures specific for each primer for 1 min and extension at 72ºC /45s. Final elongation was at 72°C for 10 min.

The amplified PCR fragments were separated on 8% polyacrylamide gel using vertical electrophoresis system (Mini Protean Tetra-Cell BioRad) for 50 min at 50 mA. After staining with 0.5 μg/μL ethidium bromide gels were photographed under UV light on BioDocAnalyseBiometra. The gel images of SSR data results were scored using GelAnalyser (Version 2010a) according to 20 bp ladder (Thermo Scientific).

Data analysis

Inputs for the statistical processing of the genetic analyses were prepared (converted) in the software PGDSpider version 2.1.1.3. (LISCHER and EXCOFFER, 2017). Polymorphic information content (PIC) was computed for each locus using Cervus software version 3.0.7 (KALINOWSKI *et al*., 2007). The GenAlEx 6.5 software package was used to determine the parameters of genetic variability (PEAKALL and SMOUSE, 2012). Software FSTAT 2.9.3 (GOUDET, 2001) was used for "allelic richness". To examine the presence of zero alleles in the obtained set of microsatellite data, the program Micro-Checker 2.2.3 was used. (VAN OOSTERHOUT *et al*., 2004). For the purpose of analysing the existence of a genetic bottleneck the software BOTTLENECK version 1.2.0.1. was used (PIRY *et al*., 2004).

The STRUCTURE 2.3.4. (PRITCHARD *et al*., 2000) software package based on the Bayesian cluster method was used to analyse the genetic structure of populations. The "burn-in" period was set at 10,000 iterations, while the Markov chain Monte Carlo (MCMC) number of iterations after the burn-in period was 10,000. The "admixture ancestry" model was used in combination with the "correlated allele frequency" model. The K value was set to 1-4, and for each K value the number of independent replications was 10. The analysis also included information on the individual's affiliation to a particular population, while other parameters were set by default.

RESULTS

The polymorphism was assessed using seven SSR markers on 46 sessile oak test trees from two populations. All loci were polymorphic with a high mean PIC (0.934) (Table 3). A large number of alleles was found with a total number of 127 alleles, ranging from 15 (QpZAG1 / 2) to 23 (QpZAG110) alleles per locus. The number of effective alleles ranges from 8.273 (QpZAG1 / 5) to 13.830 (QrZAG108). Gene flow between populations in the generation was calculated based on Fst values using the formula Nm = 0.25 * (1-FST) / FST. With a high flow rate, the Fst gene was low. The obtained value showed us the number of migrants in the generation between populations, and the total value was 8.522 (Table 3). Overall genetic diversity was high (He = 0.909) and varied from 0.879 to 0.928 (Table 3). Due to the excess of homozygotes observed at most loci, a significant inbreeding coefficient was detected (Fis = 0.796).

Table 3. Average genetic parameters of the studied populations

LOCUS	PIC	N	Ne	Ho	He	Fis	Fst	Nm
QpZAG110	0.944	23	11.056	0.402	0.908	0.583	0.050	5.548
QpZAG15	0.933	16	10.695	0.088	0.907	0.908	0.024	7.207
QpZAG1/2	0.918	15	10.960	0.188	0.908	0.793	-0.007	14.876
QpZAG3/64	0.953	21	12.959	0.247	0.918	0.750	0.035	6.330
QpZAG36	0.938	18	11.968	0.112	0.916	0.886	0.014	8.686
QpZAG1/5	0.899	15	8.273	0.155	0.879	0.834	0.020	7.911
QrZAG108	0.951	19	13.830	0.175	0.928	0.820	0.013	9.093
Mean	0.934	18.1	11.392	0.195	0.909	0.796	0.021	8.522

Table 4. Parameters of genetic diversity of the studied populations

Population	Locus	N	PA	Ne	Ho	He	F
I	QpZAG110	13	16	9.328	0.280	0.893	0.686
	QpZAG15	11	13	10.081	0.080	0.901	0.911
	QpZAG1/2	11	12	10.000	0.280	0.900	0.689
	QpZAG3/64	15	21	16.225	0.208	0.938	0.778
	QpZAG36	12	14	10.965	0.080	0.909	0.912
	QpZAG1/5	10	8	7.813	0.120	0.872	0.862
	QrZAG108	17	14	15.060	0.160	0.934	0.829
	Mean	12.71	14	11.353	0.173	0.907	0.810
II	QpZAG110	17	20	12.783	0.524	0.922	0.432
	QpZAG15	13	13	11.308	0.095	0.912	0.896
	QpZAG1/2	11	9	11.919	0.095	0.916	0.896
	QpZAG3/64	13	18	9.692	0.286	0.897	0.681
	QpZAG36	14	18	12.971	0.143	0.923	0.845
	QpZAG1/5	12	9	8.733	0.190	0.885	0.785
	QrZAG108	13	13	12.600	0.190	0.921	0.793
	Mean	13.29	13.71	11.429	0.218	0.911	0.761

A large amount of genetic diversity was observed in the populations I and II, with 0.907 and 0.911 He values, respectively (Table 4). When the two populations are compared, the number of alleles was higher in population II (93) compared to the population II (89). The average number of effective (Ne) alleles was higher in the population II (11.429) than in the population I (11.353) (Table 4). A high inbreeding coefficient was observed in both populations of 0.810 and 0.761, respectively (Table 4). The detected values of the index of genetic differentiation of the populations showed that there were little or moderate genetic differentiation in the research populations.

It was found that the populations are rich in unique alleles (PA). The average values by loci are 14 and 13.71, respectively (Table 4). This is a very important result that speaks of a stable population structure.

Table 5. Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Population	Locus	DF	ChiSq	Prob	Signif
I	QpZAG110	91	223,778	0.000	***
	QpZAG15	91	301,000	0.000	***
	QpZAG1/2	120	243,177	0.000	***
	QpZAG3/64	210	422,667	0.000	***
	QpZAG36	120	352,778	0.000	***
	QpZAG1/5	91	288,889	0.000	***
	QrZAG108	190	446,000	0.000	***
II	QpZAG110	210	312,585	0.000	***
	QpZAG15	120	296,333	0.000	***
	QpZAG1/2	120	296,333	0.000	***
	QpZAG3/64	78	145,833	0.000	***
	QpZAG36	136	311,640	0.000	***
	QpZAG1/5	91	212,762	0.000	***
	QrZAG108	136	268,929	0.000	***

Key: ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

Table 6. Genetic bottleneck determined by use of three mutation models

POPULATION	MUTATION MODEL					
	IAM		TMP		SMM	
	p(D)	p(E)	p(D)	p(E)	p(D)	p(E)
I	1.0000	0.00391	0.98828	0.01953	0.46875	0.59375
II	1.0000	0.00391	0.98828	0.01953	0.59375	0.46875

The results of the Hardy-Weinberg equilibrium deviation significance test showed a high level of significance for all loci in both study populations (Table 7). For all loci in the populations, the presence of null-alleles was detected by Micro-Checker 2.2.0.3. (VAN OOSTERHOUT *et al*., 2004), the studied populations were probably not in Hardy Weinberg equilibrium.

Table 6 presents the results of two methods and the Wilcoxon significance test, where p (D) is the significance of the Wilcoxon test for loss of heterozygosity in relation to heterozygosity of the population which is at the equilibrium of mutations and shifting, and p (E)

is the significance of Wilcoxon test for excess heterozygosity in relation to heterozygosity of the population which is the equilibrium of mutations and shifting. The P values lower than 0.05 were considered significant. The IAM model was not informative for SSR markers, while the test of the two-phase TMP model showed that both populations have a significant excess of expected heterozygosity compared to heterozygosity of the population at equilibrium of mutations and shifting indicating recent passage of these populations through the genetic bottleneck.

The Analysis of Molecular Variance (AMOVA) confirmed that genetic diversity was more pronounced within populations (77.5%) than among them (1.6%) (Table 7).

Table 7. Analysis of Molecular Variance (AMOVA)

Source	df	SS	Variance components	Percentage of variation
Among population		8.080	0.0528	1.6
Among individuals within population		249.464	2.498	77.5
Within individuals		31.000	0.67391	20.9
Total		288.543	3.225	

Table 8. The average allele frequency (Q) of the studied populations in the source populations

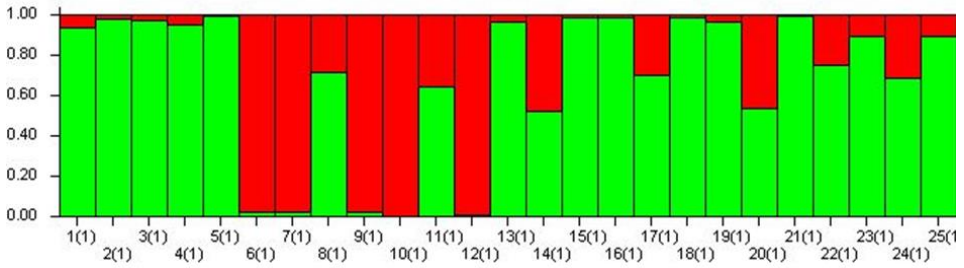
Population	K=2		K=4			
	Q1	Q2	Q1	Q2	Q3	Q4
I	0.312	0.688	0.192	0.462	0.239	0.107
II	0.331	0.669	0.379	0.009	0.157	0.455

The average allele frequency (Q) of the studied populations showed that the individuals originate from two or more populations (Table 8).

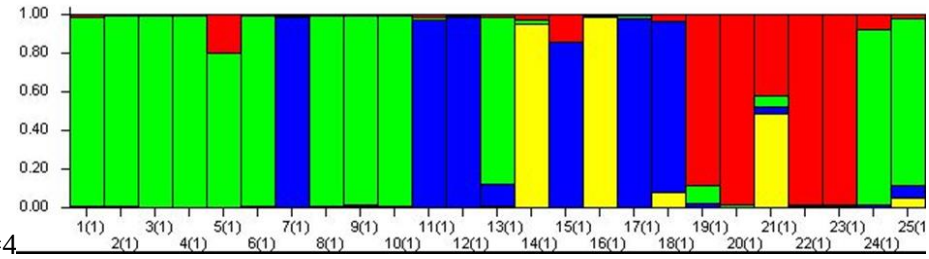
Figure 1 shows the structure of the source populations based on the Bayesian analysis using the STRUCTURE software. Based on the STRUCTURE results K = 2 was defined as the most likely number of clusters and the second most likely clustering was at K = 4. According to cluster K = 2 we can see that individuals from the population I and the population II are very similar, while according to cluster K = 4, the frequency of individuals in both populations was different.

According to our knowledge, this is the first paper whose subject is the assessment of the genetic structure of sessile oak in the area of outstanding natural beauty (AONB) "Avala". The polymorphism was assessed using seven SSR markers on 46 sessile oak test trees from the two populations. All loci were polymorphic with a high mean PIC (0.934). Markers that have already been used for the species were selected to make our results comparable to the results published in previous studies. The markers used showed a high level of genetic diversity between the analysed trees.

K=2

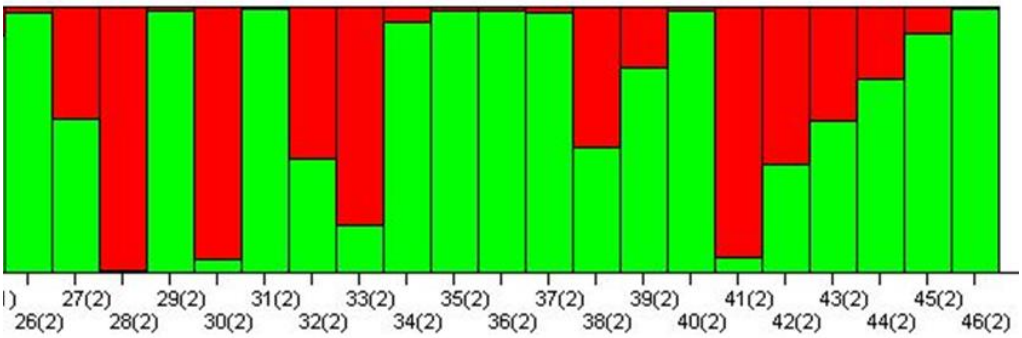


K=4

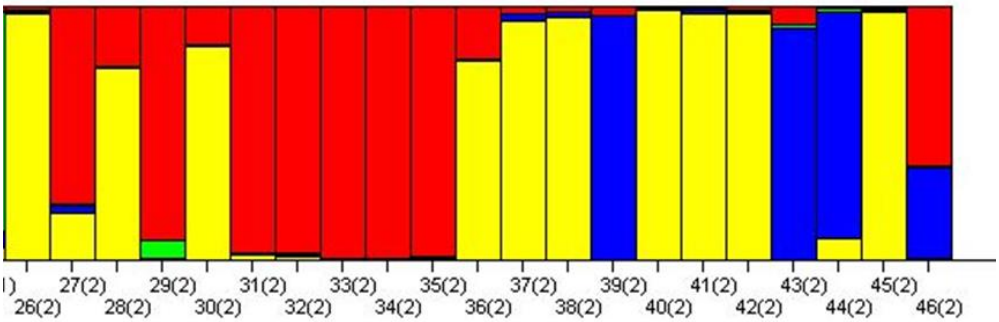


I population

K=2



K=4



II population

Figure 1. Clusters as determined by STRUCTURE at K =2 and K=4

DISCUSSION

A large number of alleles was found (127) with a mean value of 18.1 (Table 3). The number of the obtained alleles was higher than that obtained in the studies in Calabrian populations with 13.5 alleles (LUPINI *et al*., 2019) or in different Italian populations (BRUSCHI *et al*., 2003), and significantly higher than the number of alleles (12.4) obtained in populations from central Italy (ANTONECCHIA *et al*., 2015) and populations from Turkey with 12.5 alleles (YÜCEDAĞ and GAILING, 2013). That was in accordance with the highest genetic diversity in tree species among all plant species, including high percentage of polymorphic loci and large number of alleles per locus (PORTH and EL-KASSABY, 2014). The number of effective alleles (Na) with an average value of 11.392 was significantly higher than that obtained in the study with Calabrian populations (LUPINI *et al*., 2019). The high level of genetic diversity was determined (He = 0.909), which was similar to the results obtained in Calabrian populations (He= 0.749) (LUPINI *et al*., 2019). On the other hand, an excess of homozygotes (Fis = 0.796) and a low level of population differentiation (Fst = 0.021) were found. This is in line with the results published in previous studies of populations from Italy (ANTONECCHIA *et al*., 2015), France (ALBERTO *et al*., 2010), Greece, Germany, Turkey (ALBERTO *et al*., 2013; YÜCEDAĞ and GAILING, 2013), Romania (CRACIUNESC *et al*., 2016) and Calabria (LUPINI *et al*., 2019). Decreased heterozygosity within two populations may be due to the presence of a subpopulation with different allele frequencies which reduces the overall heterozygosity of the population (ALENDORF and LUIKART, 2007). This is also the case with the studied populations that are located nearby, only on different slopes of the mountain. High kinship was a consequence of gene flow as determined in the previous researches on oak species (HOKANSON *et al*., 1993; LIND-RIEHL, 2014). The loss of heterozygosity may be a consequence of self-fertilization within populations or genetic differentiation between populations (MORIĆ, 2016). Positive values of the Fis and Fit coefficients indicate differentiation, and negative values indicate an excess of heterozygotes (ANĐELKOVIĆ and RADAK, 2013). Low values of the Fst coefficient indicate similarity of allele frequency within the population, while higher values indicate difference in allele frequency (HOLSINGER and WEIR, 2009). All these results confirmed the level of genetic diversity in wind-pollinated woody species (HAMRICK and GODT, 1990).

The highest percentage of variability was determined within the population (77.5%) (Table 7). This has been confirmed in the studies with *Quercus* spp. populations (GONZALEZ-RODRIGUEZ *et al*., 2005; ZHANG *et al*., 2007; ZOLFAGHARI *et al*., 2009; SHIRAN *et al*., 2011; CRACIUNESC *et al*., 2016). LUPINI *et al*. (2019) investigated genetic structure of sessile oak forests in Calabria, Italy. They applied six nuclear SSR and four chloroplast SSR loci. The analysis of molecular variance (AMOVA) highlighted a significant higher estimated variance within populations compared to between populations. The population differentiation was not statistically significant (Fst = 0.131) and the share of genetic variation between populations was low (1.6%) (Table 7). Low values of the Fst coefficient were observed in the Balkan populations *Q. robur* (0.039) and *Q. petraea* (0.049) (NEOPHYTOU *et al*., 2010) and the Calabrian populations *Q. petraea* (0.072) (LUPINI *et al*., 2019). The results of our research were in line with research conducted on other oaks (HOKANSON *et al*., 1993; CURTU *et al*., 2007). The determined variability between individuals was a consequence of gene flow due to the formation of oak

forest complexes in a relatively small area, and information is very useful when adopting conservation strategies and proposing management measures.

CONCLUSIONS

The analysis of sessile oak genetic diversity using seven SSR markers showed a significant allelic diversity. All loci were polymorphic with a high mean value. The total number of alleles was 127 with an average of 18.1 alleles per locus. The observed heterozygosity was lower than the expected heterozygosity, which resulted in a positive value of the fixation index and an excess of homozygosity. Intra-population variability was significantly greater than inter-population variability.

Given the obtained results, the future plans for the management and restoration of this forest complex have to be made with more caution.

Large inter-individual differences point to the need to sample a large number of individuals at the population level in order to capture the genetic diversity of the species during ex-situ conservation or production of reproductive material. The conducted research is a good starting point for further researches and conservation of the gene pool of the species, but at the same time imposes the need for more intensive implementation of conservation and protection activities through *in-situ* and *ex-situ* conservation.

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GENETIČKA STRUKTURA KITNJAKA (*Quercus petraea* (Matt.) Liebl) SA PODRUČJA PIO „AVALA“

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Izvod

Cilj istraživanja bio je da se pomoću SSR markera izvrši molekularno genetička identifikacija hrasta kitnjaka sa područja PIO “Avala” te da se na osnovu dobijenih rezultata daju smernice za očuvanje raspoloživog genofonda i propišu mere gazdovanja ovim šumama. Genomska DNA je izolovana iz tkiva listova 50 test stabala iz dve populacije. Genotipizacija je izvršena sa mikrosatelit markerima QpZAG110, QpZAG15, QpZAG1/2, QpZAG3/64, QpZAG36, QpZAG1/5, QrZAG108. Svi lokusi su bili polimorfni sa visokom srednjom vrednošću PIC (0,934). Ukupan broj alela otkrivenih u istraživanihm populacijama je 127. Raspon alela varira od 15 (QpZAG1/5, QpZAG1/2) do 23 (QpZAG110), sa prosekom od 18,14 alela po lokusu. Broj efektivnih alela se kreće od 8,273 (QpZAG1/5) do 13,830 (QrZAG108). Srednja vrednost protoka (Nm) gena iznosi 8,522 sa rasponom od 5,548 do 14,876. Sveukupno genetski diverzitet je bio visok (He = 0,909) i varirao od 0,879 do 0,928. Zbog viška homozigota uočenog na većini lokusa detektovan je značajan koeficijent inbridinga (Fis = 0,796). Analiza molekularne varijanse (AMOVA) potvrdila je da je genetska raznolikost bila izraženija unutar (77,5%) nego među (1,6%) populacijama. Prosečan udeo pripadnosti (Q) istraživanih populacija pokazuje da jedinke vode poreklo od dve ili više populacija. Dobijeni rezultati mogu poslužiti za donošenje odgovarajućih planova upravljanja zaštićenim prirodnim dobrom i gazdovanjem ovom ekološki i ekonomski važnom vrstom drveća. Takođe, dobijeni rezultati omogućavaju donošenje potrebnih mera za očuvanje genetičkih resursa kitnjaka metodama in situ i ex situ. Na osnovu rezultata istraživanja mogu se dati preporuke za korišćenje ove značajne vrste u njenoj reintrodukciji u optimalnim mikroklimatskim uslovima, kao i u selekciji najboljih individua za proizvodnju reproduktivnog materijala.

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