Izolacija i karakterizacija mikrosatelitnih biljega ljekovite kadulje (Salvia officinalis L., Lamiaceae)

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IZOLACIJA I KARAKTERIZACIJA MIKROSATELITNIH BILJEGA LJEKOVITE KADULJE (*Salvia officinalis* L., LAMIACEAE)

DOKTORSKI RAD

Zagreb, 2012.

UNIVERSITY OF ZAGREB
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Ivan Radosavljević

ISOLATION AND CHARACTERIZATION OF MICROSATELLITE MARKERS IN COMMON SAGE (*Salvia officinalis* L., LAMIACEAE)

DOCTORAL THESIS

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Ovaj je doktorski rad izrađen u Botaničkom zavodu Biološkog odsjeka Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu, pod vodstvom prof.dr.sc. Zlatka Libera. Istraživanja su provedena u sklopu znanstvenog projekta "Filogenija i genetska raznolikost endemičnih biljaka dinarsko-jadranskog krša" (šifra projekta: 119-1191193-1232), financiranog od strane Ministarstva znanosti i športa Republike Hrvatske, a u sklopu Sveučilišnog poslijediplomskog doktorskog studija Biologije pri Biološkom odsjeku Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu. Dio istraživanja je sufinanciran od strane projekata "Genetic Structure of Dalmatian Sage (*Salvia officinalis* L.) Populations: A Model for a Collaborative Research on MAP Genetic Resources (SEEDNet)'', "Bioraznolikost ljekovitog i aromatičnog bilja (šifra projekta: 178-1191193-0212, MZOŠ)", "Kmetijske rastline – genetika in sodobne tehnologije " (šifra projekta: P4 – 0077, Ministrstvo za visoko šolstvo, znanost in tehnologijo, Republika Slovenija).

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Izolacija i karakterizacija mikrosatelitnih biljega ljekovite kadulje (*Salvia officinalis* L.; Lamiaceae)

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Cilj ove disertacije bio je razvijanje i primjena mikrosatelitnih biljega na populacijama ljekovite kadulje (*Salvia officinalis* L.). Isto tako nastojalo se primijeniti razvijene biljege na drugim vrstama roda *Salvia* L. te vrsti *Rosmarinus officinalis* L.. Razvijeno je ukupno 29 biljega, a primjenom osam najinformativnijih provedena je populacijsko-genetička analiza 45 populacija ljekovite kadulje s Balkanskog poluotoka. Srodstveni odnosi između većine populacija bili su korelirani s njihovom geografskom udaljenošću. Izuzetak je pet populacija za koje se pretpostavlja da nisu prirodne nego potječu iz uzgoja. Prema alelnom bogatstvu središte genetičke raznolikosti ljekovite kadulje nalazi se u Srednjoj i Južnoj Dalmaciji. Primjena mikrosatelitnih biljega ljekovite kadulje na kratkozupčastoj kadulji (*S. brachyodon* Vandas), otkrila je prolaz populacije s poluotoka Pelješca kroz genetsko usko grlo. Dobiveni rezultat naglašava potrebu preispitivanja statusa ugroženosti ove endemične vrste. Mikrosatelitna analiza ljekovite i grčke kadulje na otoku Visu (*S. fruticosa* Mill.) potvrdila je postojanje hibridne svojte *S x auriculata* Mill..

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Ključne riječi: mikrosateliti, ljekovita kadulja, cross-amlifikacija, populacijska genetika

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BASIC DOCUMENTATION CARD

University of Zagreb
Faculty of Science
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Doctoral thesis

Isolation and characterization of microsatellite markers in common sage (*Salvia officinalis* L.; Lamiaceae)

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The goal of this dissertation was development and application of microsatellite markers on common sage populations, as well as their application on other sage species and *Rosmarinus officinalis* L. Overall, 29 markers were developed, and by using eight of highest informativity, population genetic analysis of 45 common sage populations from Balkan peninsula was performed. Relationships between most of populations were corelated with their geographical distribution, with exeption of 5 populations which are believed to originate from cultivation. According to allelic richness, center of common sage genetic diversity is located in Central and Southern Dalmatia. Apllication of microsatellite markers from common sage on *Salvia brachyodon* Vandas revealed a recent bottleneck event in population from Pelješac peninsula, thus stressing the need for redefining of conservation status of this endemic species.
Microsatellite analysis of common and greek sage (*S. fruticosa* Mill.) from Island of Vis confirmed presence of lineage of hybrid origin, *S x auriculata* Mill..

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Thesis deposited in Central biological library, Department of Biology, Faculty of Science, University of Zagreb – Trg Marka Marulića 20/II, HR-10000 Zagreb, Croatia

Key words: mikrosatellites, common sage, cross-amplification, population genetics

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Reviewers: Zlatko Šatović, professor
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SADRŽAJ

PRILOG 1.

South East European Development Network on Plant Genetic Resources
The SEEDNet Medicinal and Aromatic Plants (MAP) Working Group

Genetic Structure of Dalmatian Sage (*Salvia officinalis* L.) Populations: A Model for a Collaborative Research on MAP Genetic Resources

- Project Report -

2011

SEEDNet (South East European Development Network on Plant Genetic Resources)

In order to ensure a long-term conservation of it´s valuable plant genetic resources and promote for a sustainable utilisation a number of national institutions in the region establish SEEDNet (South East European Development Network on Plant Genetic Resources) in 2004 in order to strengthen the national efforts. The main objective of SEEDNet is long-term conservation and sustainable utilisation of the diversity of PGR within the region through a well co-ordinated network of functional national programmes. The network activities comprise ex and in situ conservation, utilisation of PGR, and institution and capacity building. SEEDNet operates through six crop oriented and one thematic regional working groups. All activities of the network are planned and supervised by the Regional Steering Committee. The network is financially supported by the Swedish International Development Agency (Sida). The Swedish Biodiversity Centre (CBM), Swedish University of Agricultural Sciences provides the secretariat and coordination for SEEDNet.

The SEEDNet Medicinal and Aromatic Plants (MAP) Working Group

The SEEDNet MAP WG has 12 members appointed by the SEEDNet partner institutions. The chair is Zora Dajić, Serbia.

Project title:

Genetic Structure of Dalmatian Sage (*Salvia officinalis* L.) Populations: A Model for a Collaborative Research on MAP Genetic Resources

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Genetic Structure of Dalmatian Sage (*Salvia officinalis* L.) Populations: A Model for a Collaborative Research on MAP Genetic Resources

Summary

Biochemical analysis of essential oils and DNA fingerprinting via microsatellite markers were utilized to define the extent of diveristy existing in Dalmatian sage (*Salvia officinalis* L.) populations in SEEDNet partner countries (Albania, BiH-FBiH, BiH-RS, Croatia, Bulgaria, Kosovo, Macedonia, Moldova, Montenegro, Romania, Slovenia and Serbia). Ecogeographical surveys and collecting of Dalmatian sage populations have been carried out in order to collect leaf material to be used in the analyses of essential oil composition as well as microsatellite marker diversity. Seed samples of each population were collected and stored in national collections/gene banks.

Biochemical analysis of 46 populations included the essential oil extraction and the assessment of essential oil content and composition by Gas Chromatography-Mass Spectrometry (GC/MS). A total of of 81 volatiles were identified as constituents of investigated essential oils. The 12 main constituents representing more than 10% of the total oil content in at least a single population were: camphene, β-pinene, 1,8-cineole, trans-sabinene hydrate, cis-thujone, trans-thujone, camphor, borneol, trans-caryophyllene, α-humulene, viridiflorol and manool. Considerable variation among populations was found for the composition of the essential oils. By using multivariate analyses the populations were classified into nine main chemotypes.

A total of 45 populations represented by 20 to 25 plants were included in genetic analysis by eight microsatellite markers revealing a total of 186 alleles. The allelic richness per population ranged from 2.7 to 10.4, with a mean value of 7.3. Unrooted Neighbor-joining tree based on Cavalli-Sforza's chord distance showed that the most of the populations grouped together in accordance with geographical position of their collecting sites, from Slovenia in the North-West of the Region to Macedonia in the South-East, with the exception of five populations (two from Kosovo, one from Serbia, two from Romania, and two from Moldova) that grouped separately from the rest. Having in mind that these seven samples also had a considerable lower allelic richness in comparison to the rest, it is plausible that these samples represent cultivated material (as confirmed in case of samples Serbia and Romania) or naturalized population of plants that have escaped from earlier cultivation and grow spontaneously (as confirmed in case of Moldavian samples). The results of the model-based clustering methods as implemented in STRUCTURE software were in accordance to those obtained by distance-based method.

Key words: Salvia officinalis L., essential oil composition, chemotypes, microsatellite markers, genetic diversity

1. Introduction

Efficiency of the conservation efforts depends on the amount of information on accessions held at genebank collections. As a complement to traditional use of morphological traits in characterization and evaluation, modern conservation programmes concerning medicinal and aromatic plant species include genetic and biochemical information on the accessions. The assessment of genetic and biochemical diversity is a starting point for the introduction of accessions into plant breeding programmes and agricultural production since commercial gathering could have a negative impact on biodiversity conservation.

The genus *Salvia* represents one of the largest genera in the Lamiaceae family, comprising nearly 1,000 species throughout the Old and New Worlds. Dalmatian sage (*Salvia officinalis* L.), also known as common sage or garden sage, is a perennial subshrub native to the northern coastal region of the Mediterranean and grows wild in the calcareous mountains of northern and central Spain, southern France and the western part of the Balkan Peninsula (Hedge, 1972). It is economically the most important species of the *Salvia officinalis* group (Putievsky et al. 1990) along with *Salvia fruticosa* Mill. Dalmatian sage is used as an herb with beneficial healing properties and its dried leaf (*Salviae folium*) is an authorized drug in most pharmacopoeias. Dalmatian is cultivated in the countries of the Balkan Peninsula, throughout the Mediterranean region, and in the USA. Although knowledge and use of Dalmatian sage can be dated back to the Greek Era, there is remarkable confusion concerning its taxonomy, distribution and variability.

The chemical composition of Dalmatian sage essential oils varies widely among populations (Kuštrak et al., 1984; Pitarević et al., 1984; Perry et al., 1999; Mockute et al., 2003; Zutic et al., 2003; Elementi et al., 2006; Marić et al., 2006; Bernotienė et al., 2007; Ben Farhat, 2009) and a number of classifications have been proposed in order to group different genotypes/populations into chemotypes (Tucker and Maciarello, 1990). The most commonly reported major constituents of Dalmatian sage essential oil were cis-thujone, trans-thujone, camphor and 1,8 cineaole.

Previous researches of genetic diversity and structure of Dalmatian sage in Croatia include studies of Židovec (2004) using RAPD (Random Amplified Polymorphic DNA) markers and Jug-Dujaković (2009) using AFLP (Amplified Fragment Length Polymorphism) markers. Both studies revealed high variability within the populations, while genetic differentiation among populations showed the pattern of isolation-by-distance. Recently, in the framework of the Croatian national project entitled 'Biodiversity of Medicinal and

Aromatic Plants', microsatellites, or simple sequence repeats (SSRs), have been developed for Dalmatian sage (Molecular Ecology Resources Primer Development Consortium, 2010; Radosavljević et al., 2011) and 23 populations from Croatia and two from Bosnia and Herzegovina have been analyses by eight microsatellites markers.

The objectives of this research were (A) to determine essential oil composition of Dalmatian sage populations collected in SEEDNet partner countries and to classify them into distinct chemotypes, and (B) to assess the amount and structure of population genetic diversity by microsatellite markers.

2. Material and Methods

2.1 Ecogeographical survey and collecting of Dalmatian sage populations

Ecogeographical surveys and collecting of Dalmatian sage populations have been successfully carried out in 11 SEEDNet partner countries including Albania, BiH-FBiH, BiH-RS, Bulgaria, Kosovo, Macedonia, Moldova, Montenegro, Romania, Slovenia and Serbia. At least two collecting missions were organized in each partner country in order to collect leaf material of two Dalmatian sage populations to be used in the analyses of essential oil composition as well as microsatellite marker diversity. Seed samples of each population were collected and stored in national collections/gene banks. The information on 25 Dalmatian sage populations (23 from Croatia and two from BiH-FBiH) previously analysed in the framework of the Croatian national project entitled 'Biodiversity of Medicinal and Aromatic Plants' was added.

All investigated accessions are listed in Table 1. A total of 46 populations were included in the essential oil analysis carried out by the Macedonian partner, Gjoshe Stefkov and his team at the Ss. Cyril and Methodius University, Faculty of Pharmacy in Skopje and Mihailo Ristić, IMPR Dr Josif Pančić, Beograd, Serbia. A total of 45 populations were included in microsatellite analysis. Each population had between 20 and 25 individual plants and the total number of individual samples was 1076. DNA extraction and microsatellite analyses were carried out by the Croatian partner, Zlatko Šatović and his team at the University of Zagreb, Faculty of Agriculture (Klaudija Carović-Stanko, Martina Grdiša), Faculty of Science (Zlatko Liber, Ivan Radosavljević, Danijela Greguraš) and the Institute for Adriatic Crops and Karst Reclamation, Split (Marija Jug-Dujaković). Statistical analyses of biochemical and genetic data were carried out by the Croatian partner.

Fig. 1. Ecogeographical survey and collecting of Dalmatian sage populations

Albania: Dr. Alban Ibraliu collecting Dalmatian sage populations in Rrenci Mountain

Bulgaria: Dr. Kana Varbanova collecting Dalmatian sage populations in Emona

Moldova: Dalmatian sage accessions collected and regenerated by Dr. Maria Goncearuc

Table 1. Dalmatian sage populations collected by 12 signatory partners of the SEEDNet project and included in the essential oil (EO) and microsatellite marker (MA) analysis

No.	Country/Area	Locality	Latitude (N)	Longitude (E)	EO	MA
1	Slovenia	Soligrad	45.57	13.91	SVN 1	SVN 1
2	Slovenia	Petrinje	45.57	13.90	SVN 2	SVN 2
3	Croatia	Šušnjevica	45.25	14.16	HRV 01	HRV 01
4	Croatia	Kamenjak	44.77	13.91	HRV 02	HRV 02
5	Croatia	Krk	45.23	14.57	HRV 03	HRV 03
6	Croatia	Stara Baška	44.98	14.66	HRV 04	HRV 04
7	Croatia	Cres	45.06	14.37	HRV 05	HRV 05
8	Croatia	Lošinj	44.60	14.41	HRV 06	HRV 06
9	Croatia	Vratnik	44.98	14.98	HRV 07	HRV 07
10	Croatia	Karlobag	44.52	15.10	HRV 08	HRV 08
11	Croatia	Pag	44.43	15.04	HRV 09	HRV 09
12	Croatia	Dugi Otok	44.05	15.02	HRV 10	HRV 10
13	Croatia	Otišina	44.20	15.62	HRV 11	HRV 11
14	Croatia	Pirovac	43.83	15.72	HRV 12	HRV 12
15	Croatia	Zrmanja	44.21	16.06	HRV 13	HRV 13
16	Croatia	Šparadići	43.63	15.96	HRV 14	HRV 14
17	Croatia	Vinišće	43.51	16.12	HRV 15	HRV 15
18	Croatia	Unešić	43.73	16.16	HRV 16	HRV 16
19	Croatia	Biokovo	43.40	16.92	HRV 17	HRV 17
20	Croatia	Runovići	43.36	17.27	HRV 18	HRV 18
21	Croatia	Hvar	43.13	16.95	HRV 19	HRV 19
22	Croatia	Vis	43.03	16.14	HRV 20	HRV 20
23	Croatia	Pelješac	42.98	17.27	HRV 21	HRV 21
24	Croatia	Mljet	42.75	17.51	HRV 22	HRV 22
25	Croatia	Konavle	42.60	18.25	HRV 23	HRV 23
26	BiH-FBiH	Hutovo blato	43.04	17.71	BiH-FBiH 1	BiH-FBiH 1
27	BiH-FBiH	Međine	43.34	17.75	BiH-FBiH 2	BiH-FBiH 2
28	BiH FBiH	Mostar[#]	43.33	17.75	BiH-FBiH 3	BIH-FBiH 3
29	BiH FBiH	Međugorje[#]	43.18	17.69	BiH-FBiH 4	BIH-FBiH 4
30	BiH-RS	Ljubinje	42.92	18.03	BiH-RS 1	BiH-RS 1
31	BiH-RS	Trebinje	42.71	18.40	BiH-RS 2	BiH-RS 2
32	Montenegro	Pješivci, Nikšić	42.36	19.23	-	MNE 1
33	Montenegro	Sutorman, Bar	42.15	19.12	MNE	MNE 2
34	Albania	Llogora Park	40.20	19.59	-	ALB 1
35	Albania	Rrenci Mountain	41.83	19.58	-	ALB 2
36	Macedonia	Mt. Jablanica, Globočica	41.32	20.58	MKD 1	MKD 1
37	Macedonia	Mt. Karaormar, Burinec	41.39	20.62	MKD 2	MKD 2
38	Macedonia	Galičica	41.62	20.81	MKD 3	-*
39	Kosovo	Vermicë	42.17	20.58	-	KOS 1
40	Kosovo	Mirusha	42.52	20.57	-	KOS 2
41	Serbia	Pančevo[##]	44.85	20.72	SRB 1	SRB 1
42	Serbia	Gradište	43.33	22.17	SRB 2	SRB 2
43	Romania	Bacau, Motoc[##]	46.36	27.10	ROU 1	ROU 1
44	Romania	Bacau, Motoc[##]	46.36	27.10	ROU 2	-*
45	Romania	Bacau, Motoc[##]	46.36	27.10	ROU 3	-*
46	Romania	Bacau, Motoc[##]	46.36	27.10	ROU 4	-*
47	Romania	Bihor, Avram Iancu[##]	46.67	21.53	ROU 5	ROU 2
48	Moldova	Chishinau[##]	47.36	28.85	MDA 1	MDA 1
49	Moldova	Lopatica, Calme[##]	45.95	28.41	MDA 2	MDA 2
50	Bulgaria	Emona	42.71	27.88	BGR 1	-**
51	Bulgaria	Eastern Rhodopes	41.63	25.74	BGR 2	-**

[#]Collected by Marija Jug-Dujaković, Croatia

[##]Cultivated material (SRB 1, ROU 1-5) or naturalized, non-native populations (MDA 1-2)

*Not included in microsatellite analysis

**DNA extraction failed

2.2 Essential oil extraction and GC/FID/MS analyses

The essential oils were isolated by steam-distillation in the Clevenger apparatus using method from European pharmacopoeia (Ph. Eur. 7). Essential oil samples were analyzed on Agilent 7890A Gas Chromatography system with flame ionization detector (FID), and Agilent 5975C mass spectrometer (MS) also equipped with capillary flow technology which enables simultaneous analysis of the sample on both detectors. HP-5ms (30 m x 0.25 mm, film thickness 0.25 mm) capillary column was used. Operating conditions were as follows: oven temperature 60 °C (5 min), 1 °C/min to 80 °C (2 min); 5 °C/min 280 °C (5 min); flow rate of 1ml/min (He); injector T=260 °C; FID T= 270 °C; 1ml injection volume at split ratio 1:1. The mass spectrometry conditions were: ionization voltage 70 eV, ion source temperature 230 °C, transfer line temperature 280 °C and mass range from 50-500 Da. The MS was operated in scan mode. Identification of the components present in essential oils was made by comparing mass spectra of components in essential oils with those from Nist, Wiley and Adams mass spectra libraries, by AMDIS (Automated Mass Spectral Deconvolution and Identification System) and by comparing literature and estimated Kovats retention indices that were determined using mixture of homologous series of normal alkanes from C9 to C25 in hexane, under the same above mentioned conditions.

2.3 DNA extraction and microsatellite analyses

Genomic DNA samples were extracted using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich) from dried *Salvia officinalis* L. leaves collected from 45 populations. Eight microsatellite primers were used for the analysis: SoUZ001, SoUZ002, SoUZ003, SoUZ007, SoUZ011 (Molecular Ecology Resources Primer Development Consortium et al., 2010), and SoUZ013, SoUZ014, SoUZ019 (Radosavljevic et al., 2011. Amplification was performed using a GeneAmp® PCR System 9700 (Applied Biosystems) using a two-step PCR protocol with an initial touchdown cycle. The cycling conditions were as follows: 94°C for 5 min; 5 cycles of 45 s at 94°C, 30 s at 60°C, which was lowered by 1°C in each cycle, and 90 s at 72°C; 25 cycles of 45 s at 94°C, 30 s at 55°C, and 90 s at 72°C; and 8-min extension step at 72°C. The products were run on an ABI 3730XL analyzer using the commercial GeneScan service (Macrogen). The results were analyzed using GeneMapper 4.0 software (Applied Biosystems®).

2.4 Data analysis

The relationships among the eight main essential-oil constituents were assessed by Pearson's correlation coefficient as implemented in PROC CORR in SAS (SAS Institute Inc., 2004).

Principal-Components Analysis (PCA) based on 12 main essential-oil constituents was performed using PROC PRINCOMP procedure in SAS. The biplot was constructed by two principal components showing populations and essential-oil constituents. The number of principal components was determined by checking the eigenvalues of the principal components (using the Kaiser criterion that retains components with eigenvalues >1 and SCREE plot), and the cumulative proportion of the variance explained. These principal components were further used in cluster analysis (CA).

The standardized scores of the first five principal components were multiplied by the root of their eigenvalues and the Euclidean distance matrix between all pairs of populations was calculated to be used in cluster analysis (CA). The Average linkage method (i.e. UPGMA) of PROC CLUSTER in SAS was applied in order to determine the optimal number of clusters by calculating and plotting Cubic Clustering Criterion (CCC) statistics and Pseudo F (PSF) statistics. Populations were classified into groups representing distinct chemotypes.

Polymorphism Information Content (*PIC*; Botstein *et al.*, 1980) of each microsatellite marker was calculated by PowerMarker V3.23 (Liu, 2002) software. GENEPOP 4.0 (Raymond and Rousset 1995) was used to estimate population genetic parametres (the average number of alleles per locus, N_{av}; the observed heterozygosity, H_O; the expected heterozygosity or gene diversity, H_E; inbreeding coefficient, F_{IS}) and to test population genotypic frequencies across all loci for conformance to Hardy-Weinberg (HW) expectations (multi-locus test). The allelic richness, N_{ar}, as the measure of the number of alleles per locus independent of sample size was calculated by FSTAT v. 2.9.3.2 programme package (Goudet, 1995; 2002) while the number of private alleles (N_{pr}) per population was assessed by MICROSAT (Minch et al., 1997).

Genetic differentiation between all pairs of populations was measured with pairwise F_{ST} estimates. Pairwise F_{ST} and their respective *P*-values for significant differences from zero were calculated in FSTAT. Pairwise Cavalli-Sforza's chord distance (Cavalli-Sforza and Edwards, 1967) were calculated and unrooted phylogenetic tree was constructed using Neighbor-joining algorithm with 1,000 bootstraps over microsatellite loci as implemented in

SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE programmes of the PHYLIP ver. 3.6b software package (Felsenstein, 1993).

The analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) using ARLEQUIN ver. 3.0 (Excoffier et al., 2005). AMOVA was used to partition the total microsatellite diversity among and within populations. The variance components were tested statistically by non-parametric randomisation tests using 10,000 permutations.

Isolation by distance (IBD) among populations was tested using the method of Rousset (1997). A Mantel test (10,000 permutations of population locations among all locations) on the matrix of pairwise $F_{ST}/(1-F_{ST})$ ratios and that of the natural logarithm of geographical distances (in km) between pairs of populations was performed using NTSYS-pc version 2.02 (Rohlf, 1997).

A model-based clustering method was applied to multilocus microsatellite data to infer genetic structure and to define the number of clusters (gene pools) in the dataset using the Structure v. 2.3.3 software (Pritchard et al., 2000). Given a value for the number of clusters, this method assigns individual genotypes from the entire sample to clusters in a way in which linkage disequilibrium (LD) is maximally explained. Ten runs of structure were performed by setting the number of clusters (K) from 1 to 11. Each run consisted of a burn-in period of 200,000 steps followed by 1,000,000 MCMC (Monte Carlo Markov Chain) replicates, assuming an admixture model and correlated allele frequencies. No prior information was used to define the clusters. The choice of the most likely number of clusters (K) was carried out by comparing the logarithmized probabilities of data [Pr(X|K] for each value of K (Pritchard et al. 2000), as well as by calculating an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values, as described by Evanno et al. (2005).

3. Results

3.1 Essential oil composition

Using GC/FID/MS analyses a total of 81 volatiles were identified as constituents of investigated essential oils. In each population identified compounds represented more than 85% of the oil. The 12 main constituents representing more than 10% of the total oil content in at least a single population were selected for further analysis: camphene (C01), β-pinene (C02), 1,8-cineole (C03), trans-sabinene hydrate (C04), cis-thujone (C05), trans-thujone (C06), camphor (C07), borneol (C08), trans-caryophyllene (C09), α-humulene (C10), viridiflorol (C11), and manool (C12).

A few of the main essential-oil constituents showed high intercorrelation ($r > 0.75$; Table 2). Trans-caryophyllene (C09) was highly and positively correlated with α-humulen (C10). α-humulen (C10), viridiflorol (C11) and manool (C12) were highly and positively correlated among each other.

Table 2. Pearson's correlation coefficients among the 12 main essential-oil constituents of Dalmatian sage

Compound		Compound											
		C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12
C01	camphene		**	ns	ns	ns	ns	**	*	**	***	***	***
C02	β-pinene	0.47		ns	ns	**	ns	**	ns	ns	*	**	**
C03	1,8-cineole	-0.08	-0.19		ns	ns	ns	ns	ns	ns	ns	ns	ns
C04	trans-sabinene hydrate	0.04	-0.06	0.13		ns	ns	ns	ns	ns	ns	ns	ns
C05	cis-thujone	-0.25	-0.38	0.05	-0.27		ns	*	ns	ns	ns	ns	ns
C06	trans-thujone	0.07	-0.18	0.00	-0.12	-0.01		**	ns	*	ns	ns	ns
C07	camphor	0.38	0.38	-0.10	0.15	-0.32	-0.46		ns	*	**	***	**
C08	borneol	-0.36	-0.08	0.23	-0.13	-0.22	-0.13	-0.04		ns	ns	ns	ns
C09	trans-caryophyllene	-0.39	-0.24	-0.13	0.01	-0.23	-0.29	-0.36	0.12		***	***	***
C10	α-humulene	-0.48	-0.35	-0.12	0.00	-0.22	-0.26	-0.41	0.20	0.93		***	***
C11	viridiflorol	-0.54	-0.46	0.03	-0.07	-0.03	-0.14	-0.48	0.18	0.69	0.84		***
C12	manool	-0.53	-0.43	-0.05	0.11	-0.09	-0.24	-0.39	0.22	0.67	0.81	0.87	

The significance of the correlations is indicated as follows: ***, significance at the 0.1% nominal level; **, significance at the 1% nominal level; *, significance at the 5% nominal level; ns, not significant.

The 12 main essential-oil constituents of the 46 populations of Dalmatian sage were further analyzed using principle components analysis (PCA), with five components having eighevalues higher than one and explaining 82.86% of the total variation (Table 3).

Table 3. Component loadings of the eight essential-oil constituents on the first five principal components

Compound		Principal component									
		PC1		PC2		PC3		PC4		PC5	
C01	camphene	0.688	***	0.263	ns	0.265	ns	0.201	ns	0.085	ns
C02	β-pinene	0.550	***	0.521	***	0.169	ns	-0.200	ns	0.259	ns
C03	1,8-cineole	-0.015	ns	-0.237	ns	-0.762	***	0.237	ns	0.045	ns
C04	trans-sabinene hydrate	0.015	ns	0.319	*	-0.251	ns	0.757	***	-0.356	*
C05	cis-thujone	-0.002	ns	-0.737	***	0.035	ns	-0.334	*	-0.537	***
C06	trans-thujone	0.168	ns	-0.608	***	0.247	ns	0.417	**	0.557	***
C07	camphor	0.557	***	0.592	***	-0.213	ns	-0.182	ns	-0.262	ns
C08	borneol	-0.292	*	0.114	ns	-0.641	***	-0.335	*	0.482	***
C09	trans-caryophyllene	-0.823	***	0.330	*	0.213	ns	0.014	ns	0.028	ns
C10	α-humulene	-0.922	***	0.269	ns	0.158	ns	0.014	ns	0.046	ns
C11	viridiflorol	-0.917	***	0.022	ns	0.065	ns	0.014	ns	0.006	ns
C12	manool	-0.894	***	0.148	ns	0.015	ns	0.073	ns	-0.078	ns
	Eigenvalue	4.369		1.978		1.336		1.147		1.113	
	% of Variance	36.405		16.487		11.136		9.558		9.271	

The biplot based on first two principal components, jointly explaining 52.89% of the total variation is shown in Fig. 2. The first principal components axis (PC1), explaining 36.41% of the total variation, clearly separated the four populations from Romania (ROU 1-4) showing high contents of trans-caryophyllene (C09), α-humulene (C10), viridiflorol (C11), and manool (C12), from the populations originating from BiH-FBiH, Bulgaria and Croatia, which were characterized by relatively high levels of camphene (C01), β-pinene (C02), and camphor (C07). Along the second axis, explaining 16.49% of the total variation, populations from Bulgaria, a population form Romania (ROU 5) and some populations from BiH-FBiH (BiH-FBiH 2 and 3) and Croatia, characterized by a high camphor (C07) and β-pinene (C02) content were distinguished from the rest of populations from BiH-FBiH (BiH-FBiH 1 and 4) and Croatia, which contained high cis-thujone (C05) and trans-thujone (C06) content.

Fig. 2. Biplot of the principal-components analysis based on the 12 main essential-oil constituents of 46 Dalmatian sage populations

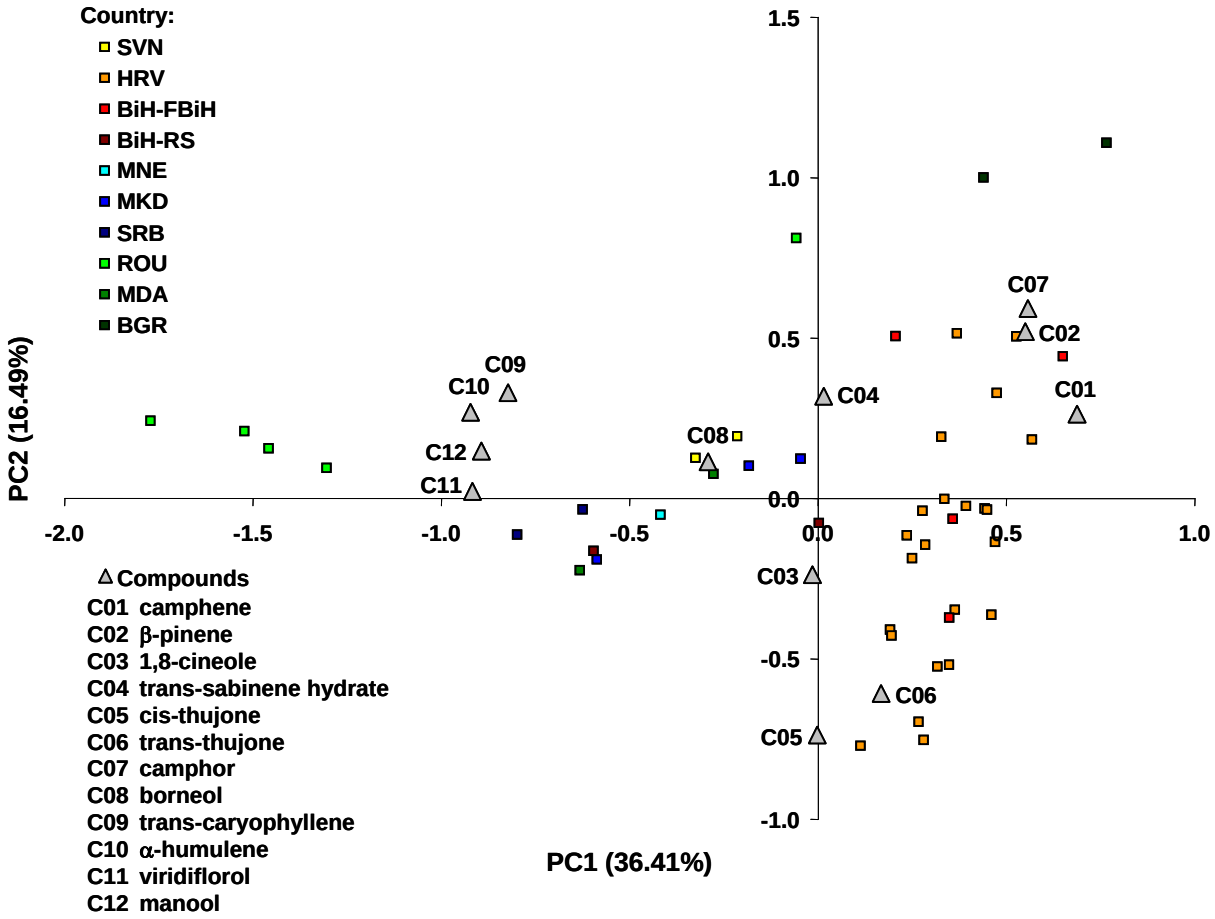
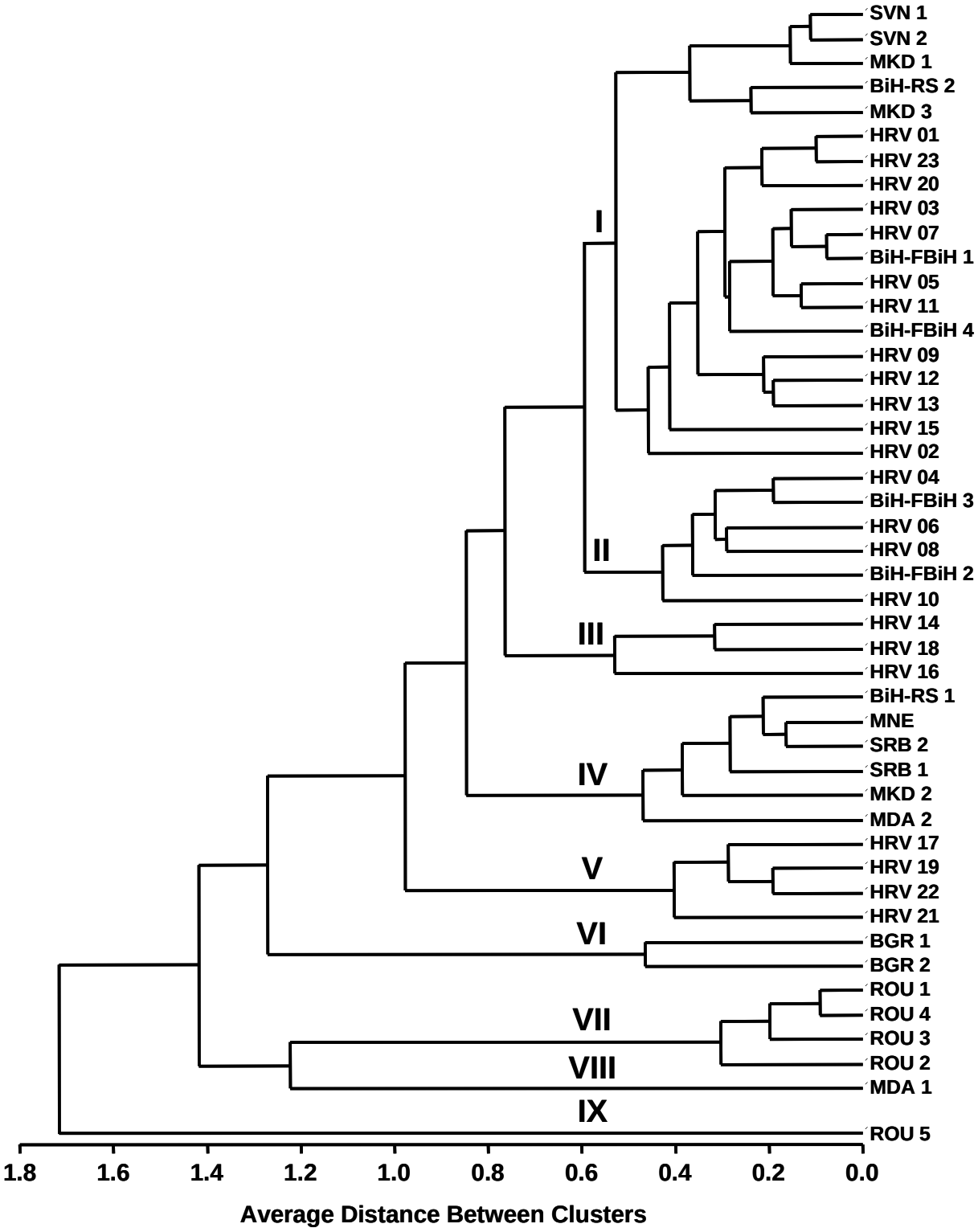


Fig. 3. UPGMA dendrogram of cluster analysis on 46 populations of Dalmatian sage using the first five principal components. Eight major clusters (I-IX) are indicated.



Cluster analysis of 46 Dalmatian sage populations based on the first five principal components resulted in the dendrogram shown in Fig. 3. The highest values of both PSF statistics and CCC were obtained for nine clusters. Thus, the classification of 46 Dalmatian sage populations into nine chemotypes was the optimal solution. Tentative classification of Dalmatian sage populations into nine chemotypes along with their description is presented in Table 4.

Table 4. The most represented compounds on average across populations belonging to different Dalmatian sage chemotypes

Chemotype	n	1	2	3	4	5
I	19	cis-thujone	camphor			
II	6	camphor	cis-thujone	1,8-cineole		
III	3	cis-thujone	1,8-cineole			
IV	6	cis-thujone	camphor	*viridiflorol*		
V	4	trans-thujone	cis-thujone			
VI	2	camphor	b-pinene	*camphene*		
VII	4	cis-thujone	a-humulene	viridiflorol	*trans-caryophyllene*	*manool*
VIII	1	cis-thujone	trans-thujone	borneol	1,8-cineole	*manool*
IX	1	camphor	trans-sabinene hydrate	1,8-cineole		

n - number of populations belonging to each chemotype
Underlined - distinctly higher average % (higher than one standard deviation above the overall mean)
Italic - less than 10% on average

3.2 Genetic diversity

A total of 186 alleles was found across the eight markers, the number of alleles per locus ranging from 13 (SoUZ013) to 39 (SoUZ001), with a mean value of 23.25 alleles per locus (Table 5). All microsatellite loci displayed high values of PIC (from 0.678 to 0.939), permitting the identification of all the individuals analysed.

Table 5. Allelic diversity of eight microsatellite loci scored in 45 Dalmatian sage (Salvia officinalis L.) populations

No.	Locus	Repeat Motif	Size Range	N_a	PIC
1	SoUZ001	$(AG)_{15}$	159-221	39	0.939
2	SoUZ002	$(TG)_{11}$	177-218	20	0.779
3	SoUZ003	$(GT)_{13}$	174-216	22	0.757
4	SoUZ007	$(GT)_{11}$	138-210	15	0.678
5	SoUZ011	$(GA)_{25}$	156-212	29	0.923
6	SoUZ013	$(AAC)_8$	179-215	13	0.813
7	SoUZ014	$(AGA)_{10}$	175-244	24	0.888
8	SoUZ019	$(AGA)_{16}$	132-199	24	0.753
	Average			23.25	0.816
	Total			186	

N_a - total number of alleles

Main parameters describing intrapopulation diversity of 45 Dalmatian sage populations are shown in Table 6. The allelic richness (N_{ar}) as revealed by eight microsatellite loci in 45 Dalmatian sage populations ranged from 2.707 (SRB 1) to 10.409 (HRV 23), with a mean value of 7.277. Five populations exhibited the allelic richness lower than 5 (KOS 1, KOS 2, SRB 1, ROU 1, ROU 2, MDA 1, MDA 2) while two populations had the values of allelic richness higher than 10 (HRV 21, HRV 23). A total of 32 private alleles has been detected in 14 Dalmatian sage populations. The highest number of private alleles was observed in population from Albania (ALB 1). The observed heterozygosity (H_O) ranged from 0.313 (SRB 1) to 0.854 (SVN 2), with a mean value of 0.854, while the expected heterozygosity (H_E) ranged from 0.377 (SRB 1) to 0.847 (HRV 23), with a mean value of 0.720. The multi-locus test for conformance to Hardy-Weinberg (HW) equilibrium was significant ($P < 0.05$) in case of 12 populations. The highly significant ($P < 0.001$) excess of heterozygotes was found in two Romanian populations (ROU 1, ROU 2), while the highly significant ($P< 0.001$) deficit of heterozygotes was observe in two Croatian populations (HRV 07, HRV 22).

Table 6. Genetic diversity of 45 Dalmatian sage populations

Abbr.	Country	Population	n	N_{av}	N_{ar}	N_{pa}	H_O	H_E	F_{IS} [#]	
SVN 1	Slovenia	Soligrad	23	8.750	7.930	0	0.745	0.795	0.063	ns
SVN 2	Slovenia	Petrinje	24	9.125	8.258	0	0.854	0.795	-0.075	ns
HRV 01	Croatia	Šušnjevica	25	6.875	6.359	0	0.701	0.720	0.026	ns
HRV 02	Croatia	Kamenjak	25	7.500	6.874	0	0.702	0.700	-0.003	ns
HRV 03	Croatia	Krk	25	8.500	7.708	0	0.717	0.734	0.024	ns
HRV 04	Croatia	Stara Baška	25	8.000	7.279	0	0.761	0.749	-0.017	ns
HRV 05	Croatia	Cres	24	7.750	7.122	0	0.765	0.729	-0.049	ns
HRV 06	Croatia	Lošinj	25	8.250	7.374	1	0.680	0.708	0.039	ns
HRV 07	Croatia	Vratnik	24	8.000	7.363	0	0.674	0.771	0.126	***
HRV 08	Croatia	Karlobag	24	7.625	6.968	2	0.724	0.773	0.063	**
HRV 09	Croatia	Pag	25	8.750	7.948	0	0.754	0.759	0.008	ns
HRV 10	Croatia	Dugi Otok	24	8.625	7.644	0	0.667	0.679	0.018	ns
HRV 11	Croatia	Otišina	25	8.375	7.527	0	0.714	0.736	0.031	ns
HRV 12	Croatia	Pirovac	24	8.000	7.107	0	0.707	0.708	0.002	ns
HRV 13	Croatia	Zrmanja	24	8.375	7.689	0	0.688	0.751	0.084	ns
HRV 14	Croatia	Šparadići	24	10.000	9.160	2	0.698	0.743	0.061	ns
HRV 15	Croatia	Vinišće	25	9.625	8.646	0	0.735	0.785	0.063	ns
HRV 16	Croatia	Unešić	25	10.000	8.990	0	0.736	0.771	0.045	ns
HRV 17	Croatia	Biokovo	24	8.875	8.189	0	0.754	0.764	0.013	ns
HRV 18	Croatia	Runovići	24	10.000	9.014	1	0.734	0.781	0.059	ns
HRV 19	Croatia	Hvar	25	10.375	9.144	1	0.745	0.755	0.013	ns
HRV 20	Croatia	Vis	24	7.125	6.655	0	0.717	0.712	-0.007	ns
HRV 21	Croatia	Pelješac	25	11.500	10.091	3	0.754	0.769	0.019	ns
HRV 22	Croatia	Mljet	25	9.500	8.685	2	0.643	0.761	0.155	***
HRV 23	Croatia	Konavle	25	11.500	10.409	0	0.825	0.847	0.026	*
BiH-FBiH 1	BiH-FBiH	Hutovo blato	24	8.125	7.477	0	0.817	0.758	-0.077	ns
BiH-FBiH 2	BiH-FBiH	Međine	21	6.625	6.313	0	0.826	0.744	-0.111	**
BIH-FBiH 3	BiH-FBiH	Mostar	25	10.625	9.709	1	0.762	0.796	0.043	**
BIH-FBiH 4	BiH-FBiH	Medjugorje	25	10.500	9.471	1	0.818	0.815	-0.004	ns
BiH-RS 1	BiH-RS	Ljubinje	24	9.625	8.866	0	0.746	0.800	0.068	**
BiH-RS 2	BiH-RS	Trebinje	22	10.625	9.847	2	0.777	0.833	0.067	ns
MNE 1	Montenegro	Nikšić	24	7.375	6.721	0	0.693	0.745	0.070	ns
MNE 2	Montenegro	Bar	22	8.375	7.651	1	0.699	0.718	0.026	ns
ALB 1	Albania	Llogora	24	9.875	8.967	10	0.744	0.766	0.029	ns
ALB 2	Albania	Rrenci	23	9.625	8.694	0	0.743	0.802	0.073	ns
MKD 1	Macedonia	Globočica	24	7.250	6.458	3	0.683	0.699	0.024	ns
MKD 2	Macedonia	Burinec	22	6.625	6.210	2	0.720	0.725	0.007	ns
KOS 1	Kosovo	Vermicë	23	4.250	4.089	0	0.598	0.561	-0.067	ns
KOS 2	Kosovo	Mirusha	24	4.375	4.076	0	0.505	0.561	0.100	*
SRB 1	Serbia	Pančevo	20	2.750	2.707	0	0.313	0.377	0.171	*
SRB 2	Serbia	Gradište	24	5.625	5.256	0	0.537	0.612	0.123	**
ROU 1	Romania	Motoc	24	4.250	4.189	0	0.783	0.610	-0.283	***
ROU 2	Romania	Bihor	22	3.750	3.639	0	0.793	0.612	-0.296	***
MDA 1	Moldova	Chishinau	23	4.125	3.886	0	0.592	0.595	0.004	ns
MDA 2	Moldova	Lopatica	24	3.250	3.121	0	0.545	0.497	-0.096	ns
	Average			7.969	7.277		0.709	0.720	0.015	
	Min			2.750	2.707		0.313	0.377	-0.296	
	Max			11.500	10.409		0.854	0.847	0.171	

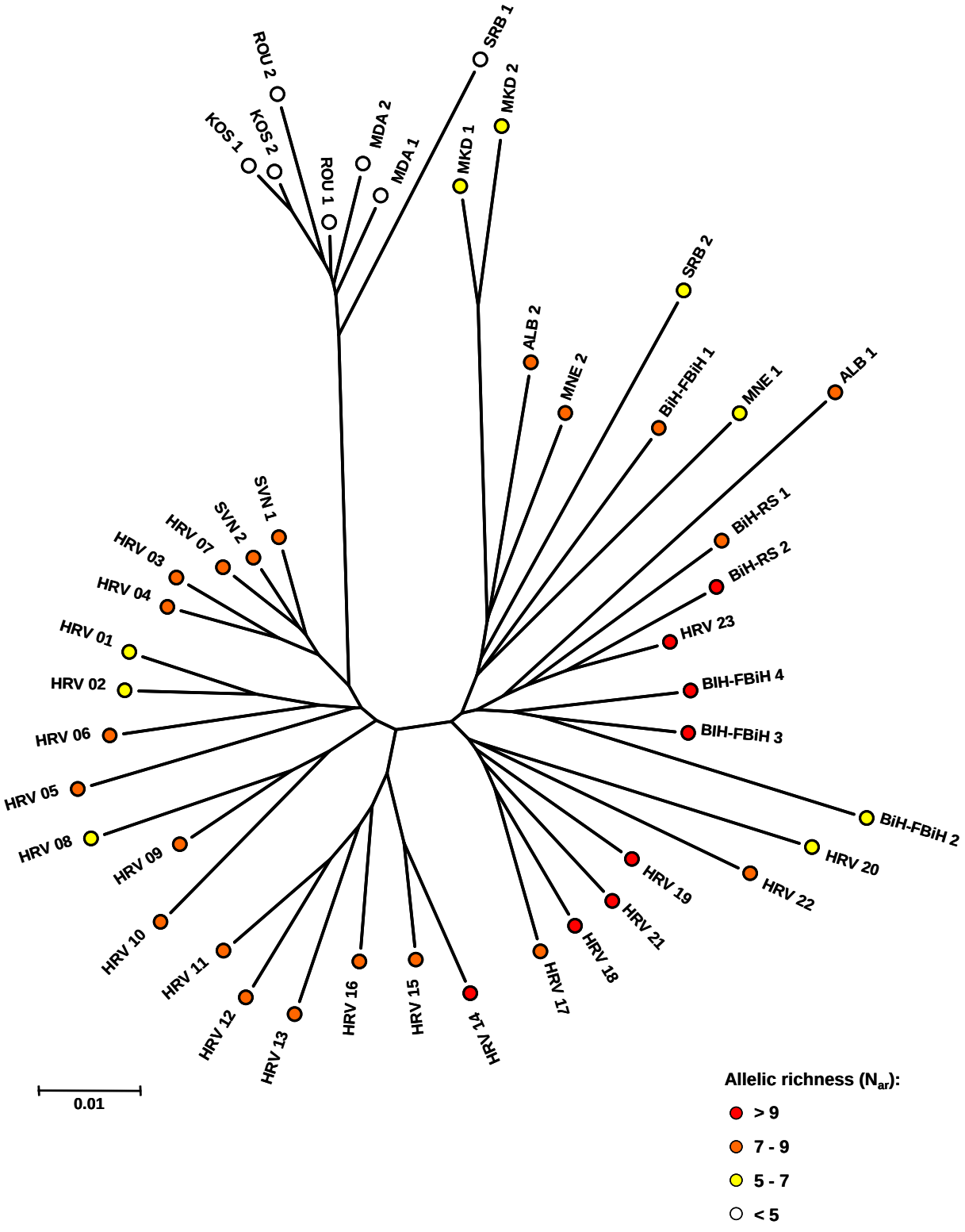
n - sample size; N_{av} - average number of alleles; N_{ar} - average number of alleles per locus independent of sample size (allelic richness); N_{pr} - total number of private alleles; H_O - observed heterozygosity; H_E - expected heterozygosity; F_{IS} - inbreeding coefficient.

[#]Probabilities of heterozygote deficiency/excess: "**" corresponds to significance at the 1% nominal level, "*" significance at the 5% nominal level and "ns" depicts non-significant values

The lowest value of genetic differentiation (F_{ST}) was observed between two Slovenian populations (SVN 1 / SVN 2; 0.003) while the highest value was found between two Serbian populations (SRB 1 / SRB 2; 0.412). Out of 990 tests of pairwise genetic differentiation, only nine were not significant: (1) SVN 1 / SVN 2, (2) SVN 1 / HRV 07, (3) SVN 2 / HRV 07, (4) HRV 03 / HRV 04, (5) HRV 03 / HRV 07, (6) HRV 14 / HRV 15, (7) HRV 23 / BiH-RS 2, (8) KOS 1 / KOS 2, (9) KOS 1 / ROU 1.

The average Cavalli-Sforza's chord distance between pairs of populations was 0.067 ranging from 0.008 between two populations from Kosovo (KOS 1 / KOS 2) to 0.131 between two populations from Serbia (SRB 1 / SRB 2). Unrooted Neighbor-joining tree based on Cavalli-Sforza's chord distance between 45 Dalmatian sage populations is shown in Fig. 4. The most of the populations grouped together in accordance with geographical position of the collecting sites, from Slovenia in the North-West of the Region to Macedonia in the South-East, with the exception of seven populations (KOS 1, KOS 2, SRB 1, ROU 1, ROU 2, MDA 1, MDA 2) that grouped separately from the rest. Having in mind that these seven samples also have a considerable lower allelic richness in comparison to the rest, it is plausible that these samples represent cultivated material (as confirmed in case of sample SRB 1 that represents the cultivated material produced by the Institute for Medicinal Plant Research "Dr Josif Pančić", Serbia as well as in case of samples ROU 1 and 2 representing landraces traditionally cultivated in Romania) or naturalized population of plants that have escaped from earlier cultivation and grow spontaneously (as confirmed in case of Moldavian samples, MDA 1 and MDA 2).

Fig. 4. Unrooted Neighbor-joining tree based on Cavalli-Sforza's chord distance between 45 Dalmatian sage populations. Allelic richness of each populations is indicated.



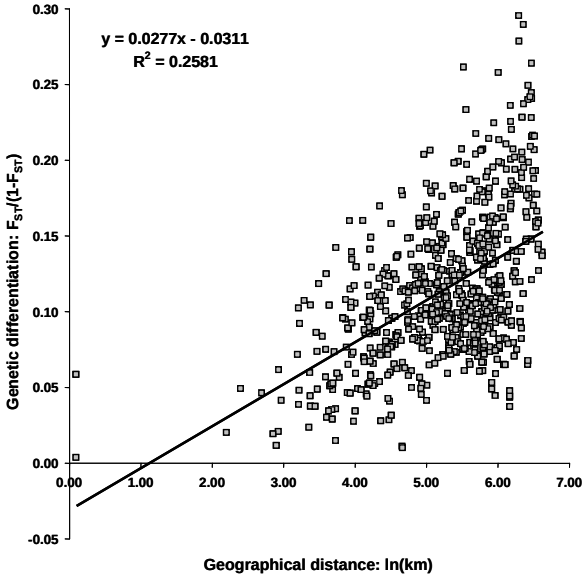
The Analysis of molecular variance (AMOVA) showed that most of total genetic diversity was attributable to differences between individuals within populations (86.63%). However, the highly significant ϕ-value of the among population component suggested the existence of genotypic differentiation (Tab. 7). By excluding seven non-native populations the within-population component of genetic diversity amounted to 89.80, but ϕ-value (0.102) was still highly significant (P < 0.0001)

Tab. 7. AMOVA analysis for the partitioning of microsatellite diversity among and between 46 Dalmatian sage populations

Source of variation	df	Variance components	% Total variance	ϕ-Statistics	P(ϕ)
Among populations	44	0.432	13.37	0.134	< 0.0001
Within populations	2107	2.802	86.63		

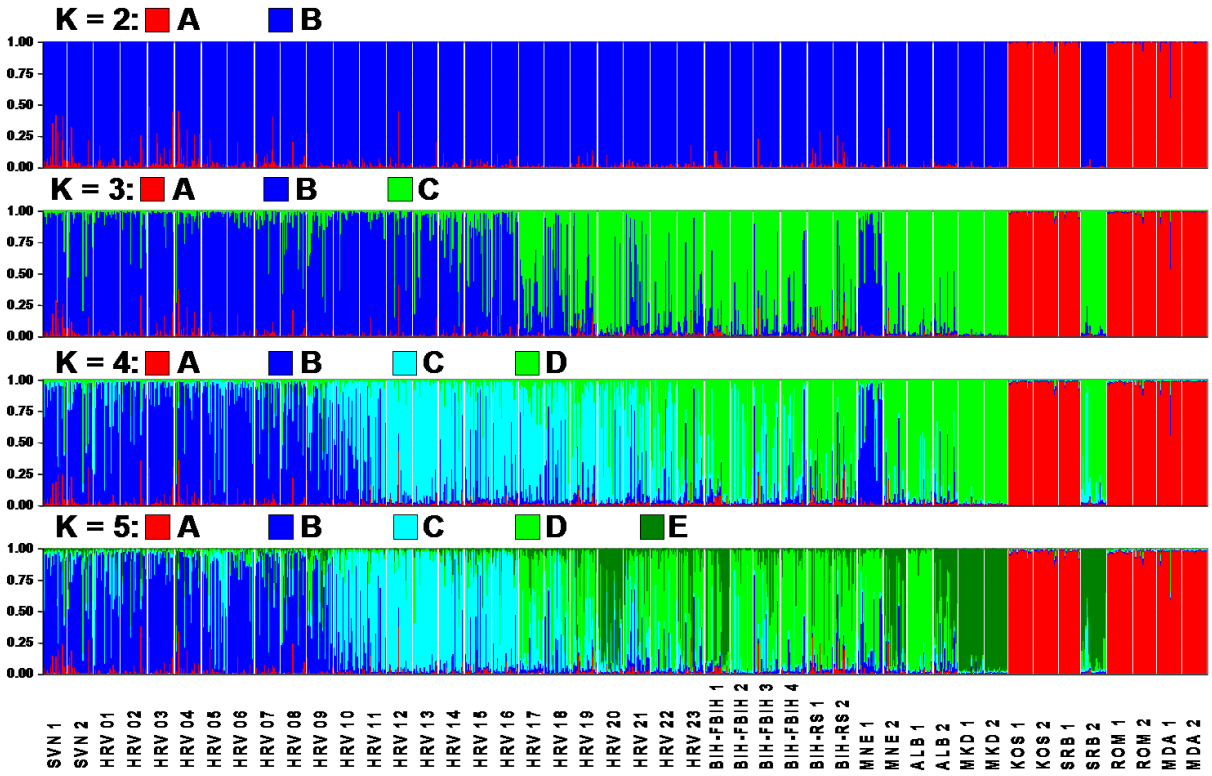
The correlation between matrices of genetic [F_{ST}/(1-F_{ST}) ratios] and geographical [ln(km)] distances was relatively high and highly significant (r = 051; P < 0.0001) indicating that 25.81% of the variance of the variance in genetic distance between populations could be explained by isolation-by-distance (Figure 5). Seven non-native populations (KOS 1, KOS 2, SRB 1, ROU 1, ROU 2, MDA 1, MDA 2) were excluded from the analysis.

Fig. 5. Isolation-by-distance among populations assessed by plotting of F_{ST}/(1-F_{ST}) ratios against the natural logarithm of geographic distances (in km) among populations.



The results of the model-based clustering methods as implemented in STRUCTURE software were in accordance to those obtained by distance-based method (Fig. 6). The proportions of membership of each individual in each cluster (i.e. gene pool) were calculated for K = 2 to 5 based on the run with the highest ln[Pr(X|K)]. At K = 2 to 5, all the individuals from seven non-native populations (KOS 1, KOS 2, SRB 1, ROU 1, ROU 2, MDA 1, MDA 2) have consistently been assigned to a separate gene pool (A). The spontaneous populations belonging to gene pool B at K = 2, split into gene pools B and C at K = 3 in accordance with geographical distribution. The same is true for further divisions at K = 4 and 5. At K = 5, the gene pool B is predominant in Slovenian populations and in Croatian populations from Northern Adriatic. The gene pool C is characteristic for Croatian populations from Middle Adriatic. The gene pool D can be found in the majority of individuals belonging to Croatian populations form Southern Adriatic (with the exception of the population HRV 20 from Vis belonging to gene pool E) as well as to BiH-FBiH and BiH-RS populations, to one population from Montenegro (MNE 1), and to one population from Albania (ALB 1). Finally, Croatian population from Vis (HRV 20), one population from Montenegro (MNE 2), one population from Albania (ALB 2), two Macedonian populations and one Serbian population (SRB 2) predominantly belong to gene pool E.

Fig. 6. Genetic structure of 45 Dalmatian sage populations. Proportions of membership for K = 2 to 5 clusters are given as estimated by Structure. Each individual plant is represented by a single vertical line divided into colours. Each colour represents one cluster, and the length of the coloured segment shows the individual's estimated proportion of membership in that cluster. White lines separate populations that are labeled below the figure



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PRILOG 2.

Tablica 1. Mikrosatelitna raznolikost populacije ljekovite kadulje (*Salvia officinalis* L.) i grčke kadulje (*Salvia fruticosa* Mill.) na otoku Visu

S.off.	Marker	N_g	N_A	R	H_O	H_E	Fis	P	
	SoUZ001	15	10	165-205	0,875	0,828	-0,057	0,950	ns
	SoUZ002	12	7	181-203	0,792	0,772	-0,026	0,288	ns
	SoUZ003	12	7	174-192	0,583	0,652	0,106	0,551	ns
	SoUZ007	11	6	196-208	0,783	0,717	-0,091	0,742	ns
	SoUZ011	11	8	166-186	0,750	0,686	-0,094	0,877	ns
	SoUZ013	10	6	182-206	0,826	0,760	-0,087	0,534	ns
	SoUZ014	15	8	175-241	0,870	0,840	-0,035	0,561	ns
	SoUZ019	6	5	132-159	0,227	0,423	0,463	0,019	*
	Mean	**11,50**	**7,13**		**0,717**	**0,712**	**-0,007**	**0,464**	**ns**
S.fru.	Marker	Ng	Nav	Range	HO	HE	Fis	P	
	SoUZ003	12	7	187-205	0,667	0,698	0,044	0,655	ns
	SoUZ005	3	3	120-150	0,125	0,198	0,367	0,025	*
	SoUZ007	3	3	202-222	0,250	0,227	-0,100	1,000	ns
	SoUZ009	3	3	209-217	0,125	0,159	0,216	0,130	ns
	SoUZ013	7	6	176-218	0,458	0,645	0,289	0,002	**
	SoUZ014	4	4	197-221	0,167	0,160	-0,040	1,000	ns
	SoUZ016	14	9	167-197	0,583	0,734	0,205	0,078	ns
	SoUZ020	4	4	198-213	0,125	0,122	-0,022	1,000	ns
	Mean	**6,25**	**4,88**		**0,313**	**0,368**	**0,151**	**0,000**	*******

N_g – broj genotipova, N_A – broj alela, R- raspon duljina umnoženih ulomaka, H_O – zapažena heterozigotnost, H_E – očekivana heterozigotnost, Fis – koeficijent samooplodnje

Tablica 2. Signifikantnost Wilcoxonovog testa za suvišak [P(E)] i nedostatak [P(D)] heterozigotnosti u odnosu na heterozigotnost populacije koja je u ravnoteži mutacija i pomaka na temelju tri mutacijska modela (IAM, TPM, SMM) kod populacije ljekovite kadulje (*Salvia officinalis* L.) i grčke kadulje (*Salvia fruticosa* Mill.) na otoku Visu

Populacija	IAM	IAM	TPM	TPM	SMM	SMM
	P(D)	P(E)	P(D)	P(E)	P(D)	P(E)
Salvia officinalis	0,809	0,230	0,320	0,727	0,014	0,990
Salvia fruticosa	0,010	0,994	0,002	1,000	0,002	1,000

IAM - model beskonačnog broja alela (*infinite allele model*; IAM); TPM - dvofazni model (*two-phase model*; TPM); SMM - model postupnih mutacija (*stepwise mutation model*; SMM)

P(E) - Signifikantnost Wilcoxonovog testa za suvišak heterozigotnosti u odnosu heterozigotnost populacije koja je u ravnoteži mutacija i pomaka ($H_E > H_{EQ}$): genetsko usko grlo

P(D) - Signifikantnost Wilcoxonovog testa za nedostatak heterozigotnosti u odnosu heterozigotnost populacije koja je u ravnoteži mutacija i pomaka ($H_E < H_{EQ}$): populacija u ekspanziji

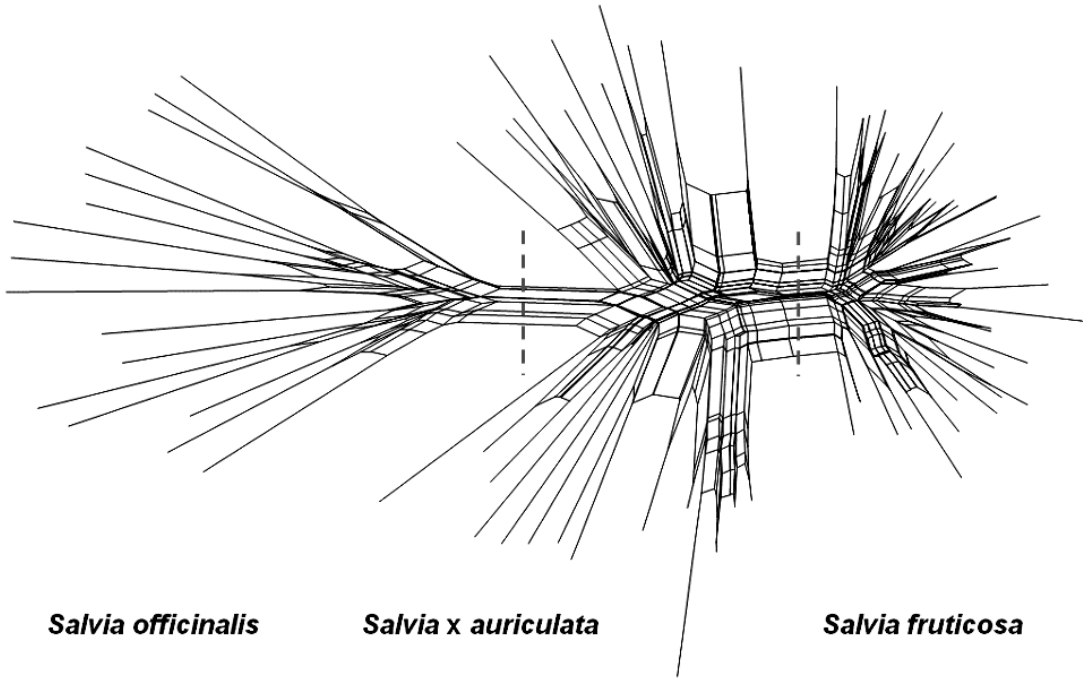
Tablica 3. Signifikantnost Wilcoxonovog testa za suvišeak [P(E)] i nedostatak [P(D)] heterozigotnosti u odnosu na heterozigotnost populacije koja je u ravnoteži mutacija i pomaka na temelju tri mutacijska modela (IAM, TPM, SMM) kod dvije populacije kratkozupčaste kadulje (*Salvia brachyodon* Vandas)

Populacija	IAM	IAM	TPM	TPM	SMM	SMM
	P(D)	P(E)	P(D)	P(E)	P(D)	P(E)
Pelješac	1.000	0.002	0.986	0.020	0.156	0.875
Orjen	0.996	0.006	0.902	0.125	0.422	0.629

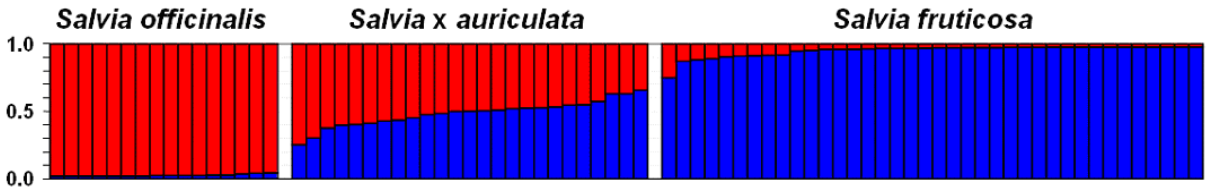
IAM - model beskonačnog broja alela (*infinite allele model*; IAM); TPM - dvofazni model (*two-phase model*; TPM); SMM - model postupnih mutacija (*stepwise mutation model*; SMM)

P(E) - Signifikantnost Wilcoxonovog testa za suvišak heterozigotnosti u odnosu heterozigotnost populacije koja je u ravnoteži mutacija i pomaka ($H_E > H_{EQ}$): genetsko usko grlo

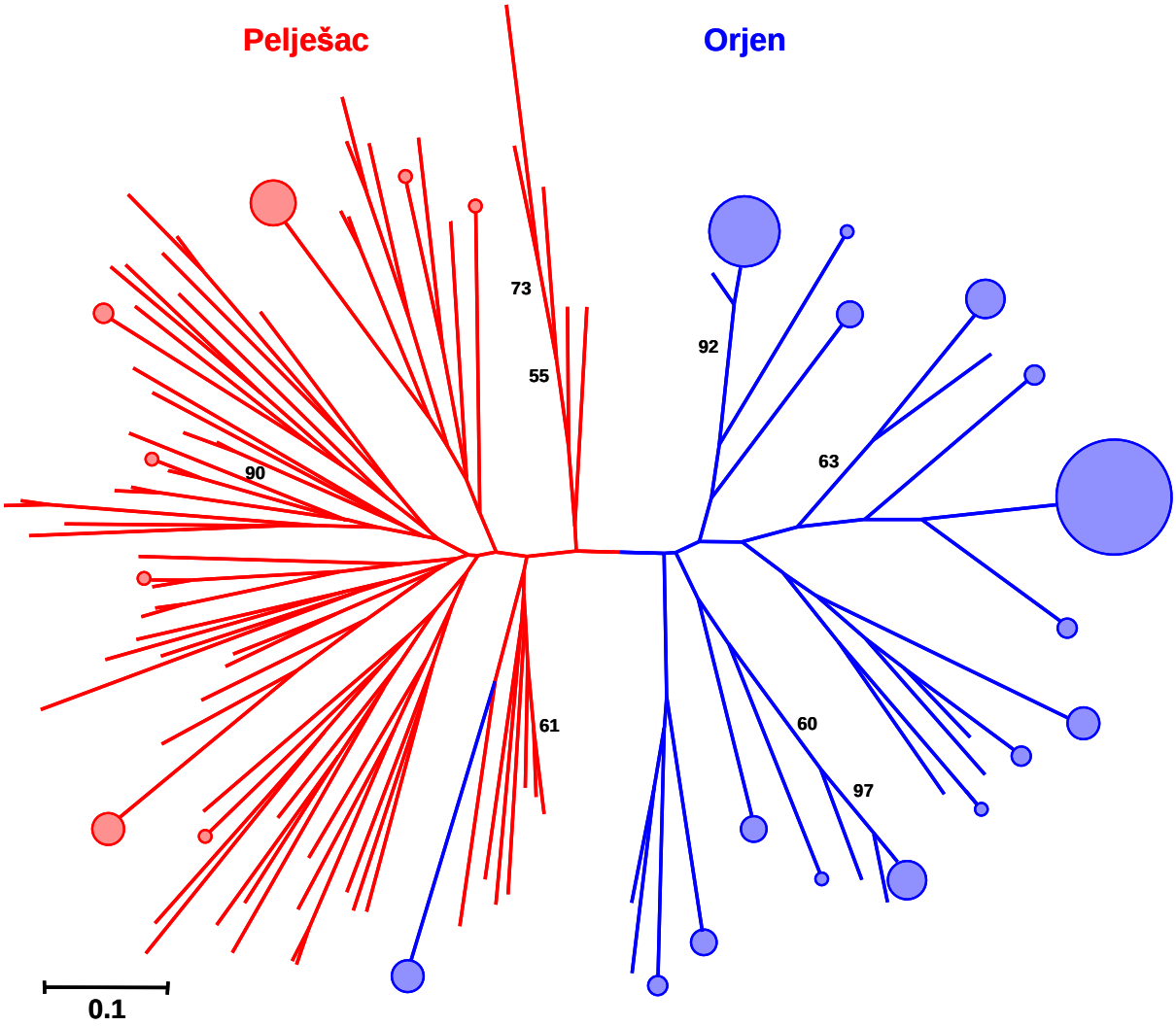
P(D) - Signifikantnost Wilcoxonovog testa za nedostatak heterozigotnosti u odnosu heterozigotnost populacije koja je u ravnoteži mutacija i pomaka ($H_E < H_{EQ}$): populacija u ekspanziji



Slika 1. Neighbour Net diagram 79 istraživanih jedinki *S.officinalis, S. fruticosa* i njihovog potencijalnog križanca na otoku Visu



Slika 2. Struktura izvornih populacija na temelju Bayesovske analize pomoću programa STRUCTURE pri K = 2: svaka je jedinka predstavljena stupcem, a boja odgovara postotku genoma (Q) jedinke koji potječe iz određene izvorne populacije



Slika 3. Nezakorijenjeno stablo izrađeno na temelju matrice genetske udaljenosti između 180 jedinki kratkozupčaste kadulje (*Salvia brachyodon* Vandas). Vrijednosti boostrap veće od 50% dobivene na temelju 1000 pseudoponavljanja označene su na pojedinim granama

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