Genetic Diversity of Dalmatian Sage (*Salvia officinalis* L.) as Assessed by RAPD Markers

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Summary

Dalmatian or common sage (*Salvia officinalis* L.) is an outcrossing plant species native to East Adriatic coast. Random Amplified Polymorphic DNA markers (RAPD) were used to analyze genetic diversity and structure of ten natural populations from the East-Adriatic coastal region. The highest genetic diversity was found in populations from the central and south Dalmatia, while the highest frequency down-weighted marker values were found in the northernmost populations and the southern most inland population. Although analysis of molecular variance (AMOVA) revealed that most of the genetic diversity was attributable to differences among individuals within populations, highly significant ϕ_{ST} values suggested the existence of genetic differentiation among populations.

By assuming Hardy-Weinberg equilibrium within populations, the calculated F_{ST} value among population was moderate. Bayesian model-based clustering method revealed that at $K = 2$ all individuals belonging to two northern populations were assigned to a separate cluster from the individuals belonging to the rest of the population. At $K = 3$, the newly formed cluster grouped the majority of individuals belonging to populations from central Dalmatia. The high correlation between matrices of genetic and geographical distances showed that isolation by distance may play a considerable role in overall structuring of the genetic diversity.

Key words

Salvia, population genetics, RAPDs, genetic structure, isolation by distance

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Introduction

The Mediterranean basin has a large number of endemic species and complicated patterns of community organization that make it one of the world plant biodiversity centres (Sales et al., 2001; Médail and Quézel, 1999). The *Salvia officinalis* group is a group of about dozen sage species characteristic for Mediterranean region (Hedge, 1972). Three most prominent species in this group are *S. officinalis* L., *S. fruticosa* Mill. and *S. tomentosa* Mill. Dalmatian or common sage (*S. officinalis*) is an outcrossing, insect-pollinated, long-lived subshrubby plant of the family *Lamiaceae*. *S. officinalis* is native to the east side of Adriatic (Ristić et al., 1999) and Ionian seas with a habitat reaching south into northwest Greece (Karousou et al., 2000). It is economically the most important species of *Salvia officinalis* group (Putievsky et al., 1990). It has been harvested mostly from wild populations in Croatia, Bosnia and Herzegovina, Montenegro and Albania. It is used as an herb with beneficial healing properties (Baricevic and Bartol, 2000), for aromatization in the meat industry and before the discovery of the antibiotics even for treatment of inflammatory processes in the organism (Amr and Djordjevic, 2000). The most abundant compounds of its essential oil are cis-thujone, camphor, and trans-thujone (Jug-Dujaković et al., 2012). Recently, Dalmatian sage has undergone promotion as an ornamental garden plant (Armitage, 1997) and several cultivars were developed for that purpose. Thanks to its use, Dalmatian sage is widespread from Mediterranean region all over the world. However, the genetic variability in the centre of its origin is still unknown.

The genetic structure of plant populations reflects the interactions of different processes including long-term evolutionary history of the species (shifts in distribution, habitat fragmentation, and population isolation), mutation, genetic drift, mating system, gene flow, and selection (Slatkin, 1987; Schaal et al., 1998). The genetic information about wild resources is a starting point for the setup of breeding programs and sustainable production that try to merge together various desirable traits (e.g. natural resistance and adaptability to different environmental conditions with useful morphological characters (Hennipman, 2000).

Random Amplified Polymorphic DNA (RAPD) technology is fast procedure for studying genetic diversity using polymerase chain reaction (Williams et al., 1990). The poor reproducibility in early RAPD analysis has been largely overcome through improved laboratory techniques and band scoring procedures (Nybon and Bartish, 2000). Since RAPD markers are dominant, attempts to diagnose genetic diversity have to be designed to take into account the fact that profiles are scored for the presence or absence of a single allele.

The aim of this study was to determine the RAPD diversity, population genetic structure, relationships, existence of spatial autocorrelation and isolation-by-distance in ten natural Dalmatian sage populations.

Material and methods

Plant samples were collected from 10 populations of *S. officinalis* throughout its range in the East-Adriatic coastal region including nine populations from Croatia and one from Bosnia and Herzegovina. Eight to 11 individuals were sampled from each population (Table 1., Fig. 1.).

Table 1. Sampling localities and genetic diversity of 10 Dalmatian sage (*Salvia officinalis* L.) populations

No.	Population	Latitude	Longitude	n	%P	*I*	H_E	DW
P01	Grižane	45°12' N	14°43' E	10	45.07	0.253	0.227	14.73
P02	Njivice, island of Krk	45°09' N	14°32' E	10	42.25	0.220	0.206	21.41
P03	Osor, island of Cres	44°40' N	14°21' E	10	50.70	0.264	0.227	7.64
P04	Murter, island of Murter	43°47' N	15°37' E	10	52.11	0.271	0.249	10.29
P05	Pirovac	43°49' N	15°40' E	8	52.11	0.282	0.250	5.74
P06	Žedno, island of Čiovo	43°29' N	16°18' E	9	59.15	0.295	0.262	10.25
P07	Postira, island of Brač	43°22' N	16°37' E	10	45.07	0.226	0.204	6.91
P08	Vidova gora, island of Brač	43°18' N	16°38' E	10	56.34	0.278	0.238	8.03
P09	Kljenak	43°12' N	17°16' E	11	64.79	0.307	0.257	11.17
P10	Vojno (Bosnia and Herzegovina)	43°20' N	17°48' E	10	40.85	0.219	0.212	11.83
	Average				50.84	0.262	0.233	10.80

n - sample size; %P - proportion of polymorphic loci; *I* - Shannon's information index of RAPD phenotypic diversity; H_E - Expected heterozygosity based on genotypic frequencies calculated using the Bayesian approach assuming Hardy-Weinberg equilibrium; DW - frequency down-weighted marker values

Genomic DNA extraction was carried out from young fresh leaves according to Doyle and Doyle (1990) with several modifications (Liber et al., 2002; Satovic et al., 2002). The amplification reactions were performed following the protocol of Williams et al. (1990) with minor modifications. The eight RAPD primers (OPB01, OPB04, OPB05, OPB10, OPB11, OPF10, OPJ01, OPX15; Operon Technologies®) were used. The final PCR reaction volume was 25 µl containing: 1 × PCR Buffer, 2.5 mM $MgCl_2$, 0.2 mM dNTP (Applied Biosystems®), 0.36 µM 10-base RAPD primer, 0.75 U *Taq* DNA polymerase (Eppendorf®) and 25 ng of DNA sample. DNA amplification was carried out for 40 cycles each comprising denaturation (0.45 min., 94°C), annealing (0.45 min., 36°C) and elongation (2.00 min., 72°C) in GeneAmp PCR System 2700 (Applied Biosystems®). One additional cycle of 5 min at 72°C

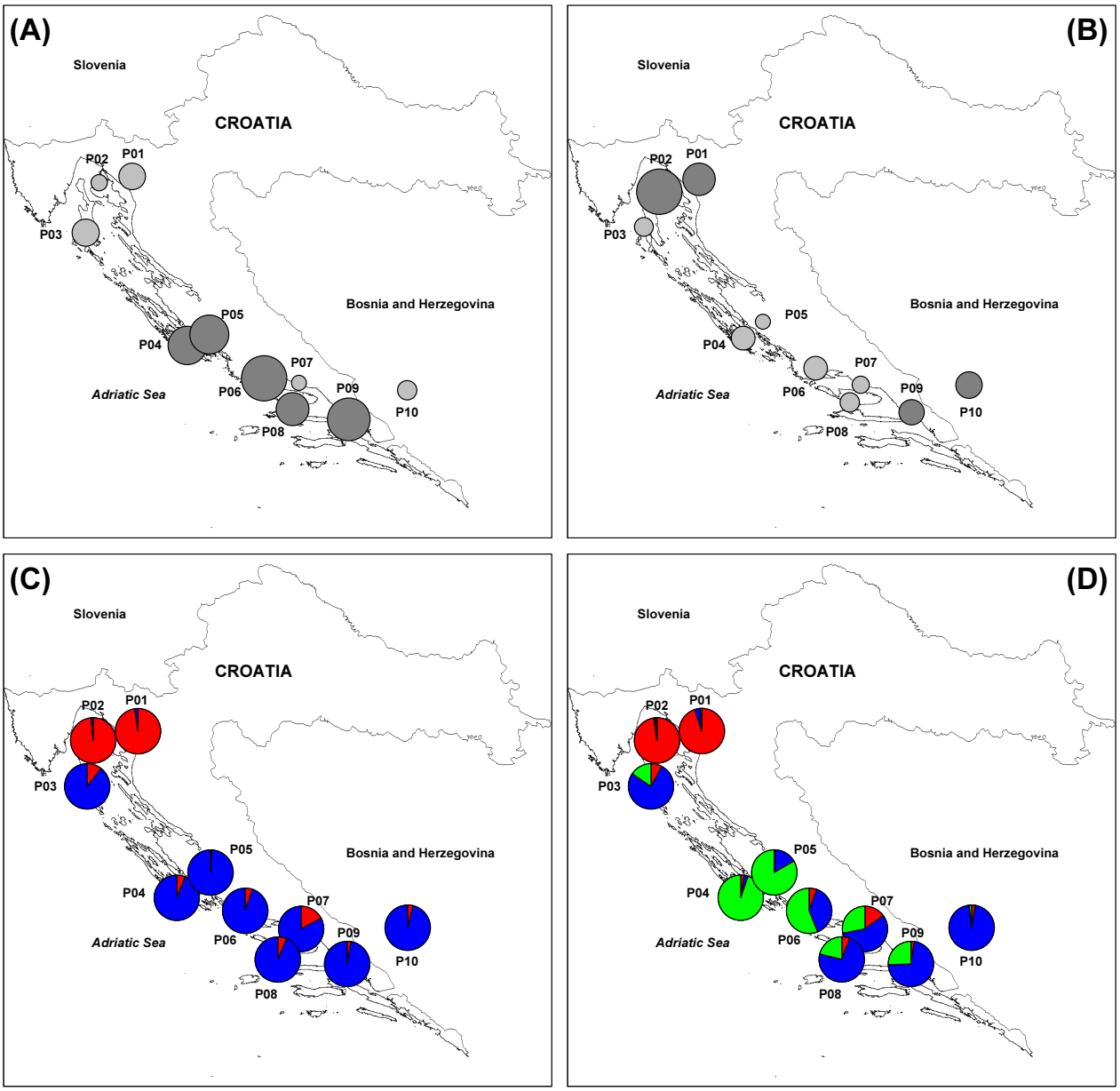

Figure 1. Genetic diversity and relationships among 10 Dalmatian sage (*Salvia officinalis* L.) populations: (A) gene diversity (H_E); (B) Frequency down-weighted marker values (DW); (C) Bayesian analysis of the population structure using the software STRUCTURE assuming $K = 2$; (D) Bayesian analysis of the population structure using the software STRUCTURE assuming $K = 3$. In (A) and (B), the size of the circles is proportional to the depicted values (dark colour represents values above average across populations and light represents values below average). In (C) and (D), the proportions of the ancestry of each population in each of the defined clusters are colour-coded (cluster A - red; cluster B - blue; and cluster C - green)

was used for final extension. The PCR products were analyzed by electrophoresis in 1.4% agarose gel, visualized by ethidium-bromide staining and photographed. The molecular weights of the fragments were determined using EZ Load™ 100 bp PCR Molecular Ruler (Bio-Rad®).

Amplified fragments were scored for the presence (1) or absence (0) of homologous bands to create a binary matrix of the different RAPD phenotypes. Shannon's information index as a measure of RAPD fragment diversity within populations was calculated as $I = -\Sigma\ (p_i\ log_2\ p_i)$, where p_i was the phenotypic frequency (Lewontin, 1972). The frequency down-weighted marker values (DW; Schönswetter and Tribsch, 2005) were calculated using AFLPdat (Ehrich, 2006).

The analysis of molecular variance (AMOVA; Excoffier et al., 1992) was used to partition the total variance into among and within populations using ARLEQUIN ver. 3.0 (Excoffier et al.,

2005). Pairwise population comparisons examined with AMOVA resulted in values of ϕ_{ST} that were equivalent to the proportion of the total variance that is partitioned between two populations. The variance components were tested statistically by non-parametric randomisation tests using 10,000 permutations.

Allelic frequencies at RAPD marker loci were calculated from the observed frequencies of fragments, using the Bayesian approach proposed by Zhivotovsky (1999) as implemented in AFLP-Surv v. 1.0 (Vekemans et al., 2002). The approach assumes Hardy-Weinberg equilibrium justified by the outcrossing nature of sage. A non-uniform prior distribution of allelic frequencies was assumed with its parameters derived from the observed distribution of fragment frequencies among loci (Zhivotovsky, 1999). As demonstrated by Krauss (2000), this approach yields almost unbiased estimates of allelic frequencies in dominant markers. Expected heterozygosity or Nei's gene diversity (Nei, 1973) and its variance were calculated for each population using the asymptotically unbiased estimator of Lynch and Milligan (1994). Allelic frequencies were also used in the analysis of genetic diversity within and between populations following the treatment of Lynch and Milligan (1994) and the calculation of pairwise Nei's genetic distances (Nei, 1972). A 1,000 distance matrices computed by bootstrapping (Felsenstein, 1985) over RAPD loci were used as input for Neighbour-joining algorithm by NEIGHBOR and CONSENSE procedures from the PHYLIP ver. 3.6b software package (Felsenstein, 1993).

A Bayesian model-based clustering method was applied on multilocus RAPD data to infer genetic structure and define the number of clusters in the dataset using the software STRUCTURE (Pritchard et al., 2000). Ten runs of STRUCTURE were done by setting the number of clusters (K) from 1 to 10. Each run consisted of a burn-in period of 200,000 steps followed by 1,000,000 MCMC (Monte Carlo Markov Chain) replicates assuming 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 examining the average estimates of the likelihood of the data conditional on a given number of clusters, $ln[Pr(X|K)]$, as suggested by Pritchard et al. (2000), and 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) and implemented in Structure-sum-2011 (Ehrich et al., 2007). The runs with the maximum likelihood were chosen and by averaging the estimated membership coefficients of the individuals, the proportion of ancestry of each population in each of the clusters was calculated. The analysis of the average genetic admixture of the populations was carried out by comparing the proportions of genome from each population assigned to the best-scoring cluster (Q_{MAX}). Thus, the populations having $Q_{MAX} < 0.75$ (at $K = 3$) were considered as admixted.

The relationship between genetic and geographic distances was assessed by two related methods. Isolation by distance (IBD) among populations was tested using the method of Rousset (1997). A Mantel test (1,000,000 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 for isolation by distance using NTSYS-pc version 2.02 (Rohlf, 1997). An indirect estimate of the number of migrants per generation assuming a two-dimensional stepping stone model was computed as $Nm = 1/b_{log}$ (Rousset, 1997; Heuertz et al., 2001), where b_{log} was the slope form the regression of pairwise $F_{ST}/(1-F_{ST})$ values on the logarithm of the geographical distances. The estimate of Nm assuming a two-dimensional stepping stone model was compared to the estimate of Nm assuming an island model calculated using the formula: $Nm = [0.25(1 - F_{ST})/F_{ST}]$ (Slatkin, 1987).

Autocorrelation indices for DNA analysis (AIDA) *II*'s, analogous to Moran's *I* (Sokal and Oden, 1978) was calculated for ten geographical distance classes. The ten geographical distance classes were created in such a manner that the number of pairwise comparisons in each distance class is approximately the same. This popular criterion for defining class boundaries produces favourable statistical properties (Epperson, 1993). The number of permutations for significance testing was set to 1,000 under the null hypothesis of no spatial structure (random distribution of individuals). The analysis was performed using the software package AIDA (Bertorelle and Barbujani, 1995).

Results

The eight RAPD primers generated a total of 88 fragments. The approximate sizes of the fragments ranged from 450 to 3,328 bp. Seventy-one out of 88 fragments (~80%) were found polymorphic among analyzed individuals. The number of polymorphic markers per primer varied from four (OPJ01) to 13 (OPB10) with an average of 8.9 markers per primer.

For each population the estimates of the proportion of polymorphic loci (%P), Shannon's information index (I) of RAPD phenotypic diversity, gene diversity (H_E) based on allelic frequencies and frequency down-weighted marker values (DW) are shown in Table 1.

Gene diversity varied from 0.204 (P07 Postira) to 0.262 (P06 Žedno) with the average value of 0.233, while the frequency down-weighted marker values (DW) ranged from 5.74 (P05 Pirovac) to 21.41 (P02 Njivice).

Analysis of molecular variance (AMOVA) of RAPD diversity was performed to examine the partitioning of the variation among and within populations (Table 2). Although most of the genetic diversity was attributable to differences among individuals within populations (75.31%), the highly significant ϕ_{ST} value among populations ($\phi_{ST} = 0.247$; $P < 0.0001$) provided a clear evidence for the existence of phenotypic differentiation between populations. ϕ_{ST} values between each pair of populations were highly significant ($P < 0.001$) in all cases except between geographically close populations P06 Žedno and P08 Vidova gora ($\phi_{ST} = 0.042$; $P = 0.052$) as well as between P07 Postira and P08 Vidova gora ($\phi_{ST} = 0.050$; $P = 0.020$) (Table 3).

The population genetic structure based on allele frequencies assuming Hardy-Weinberg equilibrium using a Bayesian approach was described in terms of the total gene diversity ($H_t = 0.277$), the average gene diversity within populations ($H_w = 0.233$), the average gene diversity among populations in excess of that observed within populations ($H_b = 0.044$), and Wright's F_{ST} statistics ($F_{ST} = 0.159$). As expected, the value of H_w was

Table 2. Analysis of molecular variance (AMOVA) for the partition of RAPD variation among and within 10 Dalmatian sage (*Salvia officinalis* L.) populations

Source of variation	df	Variance components	% Total variation	ϕ_{ST}	$P(\phi_{ST})$
Among populations	9	2.232	24.69	0.247	< 0.0001
Within populations	88	6.807	75.31		

$P(\phi_{ST})$ - ϕ-statistics probability level after 10,000 permutations

Table 3. Pairwise ϕ_{ST} values (lower diagonal) and its significance (upper diagonal) among 10 Dalmatian sage (*Salvia officinalis* L.) populations

No.	Population	Population									
		P01	P02	P03	P04	P05	P06	P07	P08	P09	P10
P01	Grižane	—	***	***	***	***	***	***	***	***	***
P02	Njivice, island of Krk	0.339	—	***	***	***	***	***	***	***	***
P03	Osor, island of Cres	0.292	0.314	—	***	***	***	***	***	***	***
P04	Murter, island of Murter	0.323	0.348	0.227	—	***	***	***	***	***	***
P05	Pirovac	0.324	0.415	0.212	0.140	—	***	***	***	***	***
P06	Žedno, island of Čiovo	0.267	0.293	0.105	0.134	0.141	—	***	ns	***	***
P07	Postira, island of Brač	0.288	0.325	0.185	0.158	0.226	0.082	—	*	***	***
P08	Vidova gora, island of Brač	0.285	0.310	0.122	0.220	0.184	0.042	0.050	—	***	***
P09	Kljenak	0.290	0.353	0.125	0.195	0.200	0.117	0.189	0.163	—	***
P10	Vojno (Bosnia and Herzegovina)	0.391	0.429	0.293	0.308	0.335	0.243	0.269	0.239	0.248	—

ns – non-significant value; * significant at P < 0.05; ** significant at P < 0.01; *** significant at P < 0.001

considerably greater than H_b corroborating the results from AMOVA. In spite of the fact that the most of gene diversity could be explained by within-population diversity, F_{ST} value of 0.159 (95% C.I: 0.146-0.165) indicated significant genetic differentiation among populations, ranging from 0.017 (between P06 Žedno and P08 Vidova gora) to 0.305 (between P02 Njivice and P05 Pirovac).

Nei's standard genetic distances between populations varied from 0.006 (between P06 Žedno and P08 Vidova Gora) to 0.138 (between P02 Njivice and P05 Pirovac) with an average of 0.059. Unrooted Neighbour-joining tree revealed that most of the clusters were not supported by a bootstrap value higher than 50% (Fig. 2). Only two moderately supported clusters were identified, the first consisting of two northernmost populations, Grižane (P01) and Njivice (P02) (Bootstrap support [BS] 57%), and the second comprised by two central Dalmatian populations, Murter (P04) and Pirovac (P05) (BS 77%).

Using the Bayesian model-based clustering method as implemented in STRUCTURE, average estimates of the likelihood of the data, conditional on a given number of clusters, $ln[Pr(X|K)]$, kept increasing with higher K. The highest ΔK value was observed for $K = 2$ (124.05), followed by ΔK value for $K = 3$ (83.57). At $K = 3$ both average and maximum values of $ln[Pr(X|K)]$ were higher than at $K = 2$, while the standard deviation was lower. Thus, the runs with the highest $ln[Pr(X|K)]$ values were chosen at $K = 2$ and 3, and the proportions of ancestry of each population in each of cluster were calculated by averaging the estimated membership coefficients of the individuals as presented in Fig. 1. At $K = 2$, all individuals belonging to populations Grižane (P01) and Njivice (P02) were assigned to a separate cluster (Cluster A) from individuals belonging to the rest of the populations (Cluster B). At $K = 3$, the newly formed cluster (Cluster C) grouped the majority of individuals belonging to central Dalmatian populations: Murter (P04), Pirovac (P05) and Žedno (P06). The average

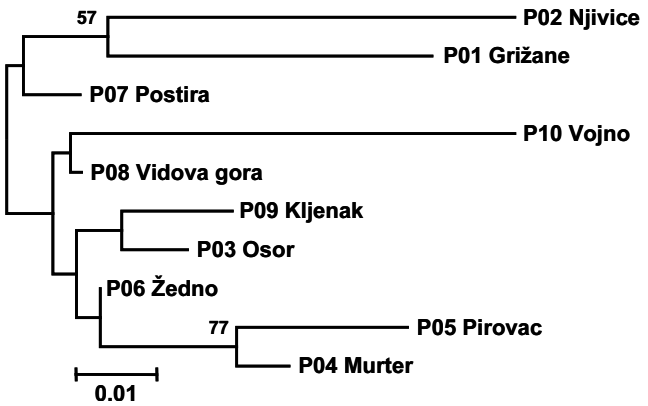

Figure 2. Neighbour-joining tree based on Nei's standard genetic distance between 10 sage populations. Bootstrap values higher than 50% obtained after 1000 permutations are indicated on the branches

admixture of the populations, expressed as the proportion of genome from a given sample assigned to the best-scoring cluster (Q_{MAX}) at $K = 3$ was 0.80 and ranged from 0.56 (P06 Žedno in Cluster C) to 0.97 (P02 Njivice in Cluster A).

The $F_{ST}/(1-F_{ST})$ ratio for pairs of populations (Lynch and Milligan's approach) increased linearly with the natural logarithm of the geographical distance (Fig. 3; $r = 0.41$; $p_{Mantel} < 0.001$; $R^2 = 0.17$;), with the relationship between genetic distance [y; $F_{ST}/(1-F_{ST})$] and geographical distance [x; ln(km)] described by $y = 0.0462x - 0.0261$ showing a typical pattern of isolation-by-distance, although explaining only 17.09% of the total variance. The estimate of the average number of migrants per generation (Nm) assuming an island model was $Nm = 1.32$ while the estimate of Nm using a two-dimensional stepping-stone model was considerably higher ($Nm = 21.64$). As the isolation-by-distance was proved significant, estimates of gene flow based on an island model is unrealistic.

Autocorrelation indices II decreased almost continuously from positive significant to negative significant as the geographical distance increased (Fig. 4) indicating non-random spatial distribution of RAPD phenotypic diversity among individuals. Significant positive values ($p < 0.01$) were obtained for the first two distance classes, where in the first class all the comparisons between individuals from the same populations (i.e. geographic distance equal to zero) were included. Significant negative values were observed for distance classes 6 to 10. The lower limit of the pairwise geographical distance included in the sixth distance class was ~100 km suggesting that at this point the isolation-by-distance may play a considerable role in overall structuring of genetic diversity.

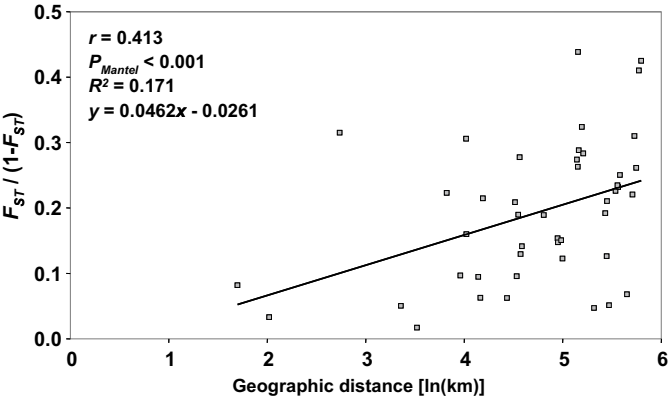

Figure 3. Isolation by distance analysis among 10 Dalmatian sage (*Salvia officinalis* L.) populations

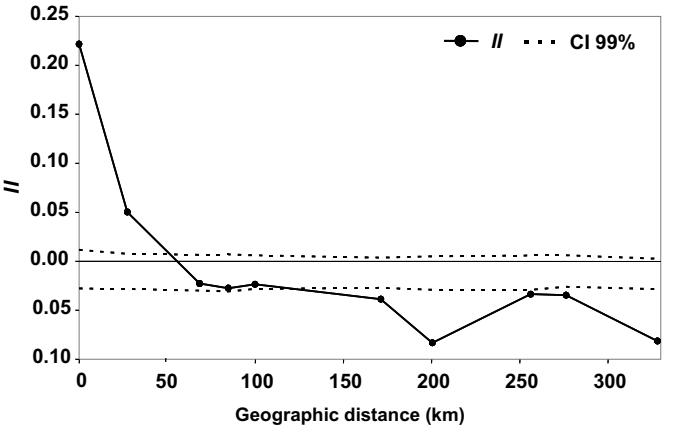

Figure 4. Correlogram of AIDA's II for ten geographical distance classes based on RAPD markers analyzed in 10 Dalmatian sage (*Salvia officinalis* L.) populations

Discussion

All analyses performed to assess genetic variability and population structure of Dalmatian sage produced similar pattern. They all indicated a high within-population diversity and significant, but moderate differentiation among populations. These results are consistent with previous results recorded in outcrossing perennial plants but not in endemic species, where much lower diversity within populations was noticed (Hamrick and Godt, 1990; Putievsky et al., 1990; Ellstrand and Elam, 1993; Fischer et al., 2000; Gitzendanner and Soltis, 2000; Nybon and Bartish, 2000; Tero et al., 2003). Dalmatian sage is an endemic species of the Apennines and the Balkan Peninsula but unlike the majority of endemic species it is widespread and often abundant. Up-to-date scientific studies support the theory about the Balkan Peninsula as Pleistocene glacial refugium for many temperate and subtropical species (Hewitt 1996, 1999; Taberlet et al., 1998; Griffiths et al., 2004). Adamovic (1908) hypothesised that *S. officinalis* was a representative of old relict flora and that its present distribution on the Balkan Peninsula was just a remnant of a much larger distribution area in the preglacial period.

Average F_{ST} of 0.159 is in the range reported in outcrossing species: 0.10-0.24 (Loveless and Hamrick, 1984), 0.099-0.216 (Hamrick and Godt, 1990), and 0.03-0.31 (Heywood, 1991). The obtained results indicate a significant level of gene flow across the entire study area of Dalmatian sage and its dependence on the geographical distance. Thus, the minimum ϕ_{ST} as well as F_{ST} values were observed between geographically neighbouring populations. It is interesting to point out that among island populations these values were not as high as expected in the case of island isolation. These results support the hypothesis that during the recent geological past Dalmatian islands were part of the mainland. Melting of glaciers and transgression at the end of Pleistocene epoch (12.000 years ago) are considered to be the reasons for an increased sea level of up to 120 meters, which resulted in submerging of the river valleys of the Adriatic basin and creation of today's Adriatic coast and islands (Velić and Malvić, 2011). In accordance with these results are the results of spatial genetic analysis that strongly emphasize the existence of isolation by distance.

The above-average gene diversity was found in the populations from the central and south Dalmatia (P04 Murter, P05 Pirovac, P06 Žedno, P08 Vidova gora, P09 Kljenak) while much lower values were observed in the northernmost populations (P01 Grižane, P02 Njivice, P03 Osor) and in the most inland

population (P10 Vojno). On the other hand, the marginal populations in the norhtern Adriatic (P01 Grižane and P02 Njivice) as well as the most inland populations (P09 Kljenak and P10 Vojno) exhibited the highest down-weighted marker values (DW). High values of DW imply a high amount of rare RAPD markers as expected for long-term historically isolated populations, whereas recently diverged populations should exhibit low DW values due to founder effects and/or genetic drift (Schönswetter and Tribsch, 2005). According to the results of STRUCTURE analysis at $K = 3$ the most admixed populations ($Q_{MAX} < 0.75$) were those form central and south Dalmatia (P06 Žedno, P07 Postira, P08 Vidova gora, P09 Kljenak) generally characterized by high gene diversity and low down-weighted marker values.

These results suggest a possible scenario in which Dalmatian sage populations survived the Last Glacial Maximum (GLM) in multiple refugia (cf. Médail and Diadema, 2009) including the possibility of several microrefugia (Rull, 2009) or cryptic refugia (Cruzan and Templeton, 2000). The central and southern Dalmatian populations, showing the highest diversity and the lowest DW values, are at the same time the most admixed populations. This region could represent the main contact zone between descendants of glacial refugia characterized by large and connected populations and unrestricted gene flow. The marginal populations in the northern Adriatic as well as the most inland populations thus represent rear-edge populations characterized by low genetic diversity, high amount of rare RAPD markers and high inter-population genetic distance.

Our results indicate that RAPD markers are sufficiently informative to assess genetic diversity of Dalmatian sage populations. In Croatia as well as Bosnia and Herzegovina and Albania Dalmatian sage is gathered in large quantities (Kathe et al., 2003). Commercial gathering in the wildness has a negative impact on biodiversity conservation (Satovic et al., 2012). In Croatia, the State Institute for Nature Protection sets yearly quotas for the collection of particular medicinal and aromatic species for commercial purposes. Quotas are set according to available knowledge concerning distribution and abundance of a particular species. With the more detailed information on population genetic structure, the quotas could be set more accurately and an appropriate *ex situ* conservation strategies could be formulated.

Conclusions

The genetic diversity and structure of ten Dalmatian sage natural populations obtained by Random Amplified Polymorphic DNA (RAPD) markers suggest the following conclusions:

1. Most of the genetic diversity was attributable to differences among individuals within populations. A high within-population diversity and significant, but moderate differentiation among populations was observed.
2. The above-average gene diversity was found in the populations from the central and south Dalmatia while much lower values were observed in the northernmost populations and in the most inland population. On the other hand, the marginal populations in the northern Adriatic as well as the most inland populations exhibited the highest down-weighted marker values (DW).
3. The results suggest a possible scenario in which Dalmatian sage populations survived the Last Glacial Maximum (GLM) in multiple refugia. The central and southern Dalmatian populations, showing the highest diversity and the lowest DW values, are at the same time the most admixed populations. This region could represent the main contact zone between descendants of glacial refugia characterized by large and connected populations and unrestricted gene flow.
4. A typical pattern of isolation-by-distance was detected.

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