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Preliminary study on genetic diversity in *Moehringia jankae* Griseb. ex Janka based on inter-simple sequence repeat (ISSR) markers

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Abstract

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Genetic diversity among eleven natural populations of the *Moehringia jankae* species, *Caryophyllaceae* family, spread in the Sinite Kamani Natural Park in Bulgaria was assessed by inter-simple sequence repeats (ISSR) markers. A total of 68 loci were identified by using 5 selected ISSR primers, of which 59 are polymorphic and 9 – monomorphic. Presences of 3 specific ISSR bands were reported in the Mj1, Mj5 and Mj9 population. For the primers used, mean values of the polymorphic information content (0.64), effective multiplex ratio (4.53), resolving power (27.66) and marker index (2.84) have been calculated. Shannon's information index (I = 0.226), expected (He = 0.149) and unbiased expected (uH = 0.165) heterozygosity, estimated based on molecular characteristics demonstrate a significant level of intra-species genetic diversity of *M. jankae*. Cluster analysis, Principal component analysis **(**PCA) and Principal coordinate analysis (PCoA) divide the studied plant populations into 2 main clusters – first (A) comprises Mj7, Mj8 and Mj10, and Mj1, Mj11, Mj9, second one (B) consists of 2 subclusters, too with Mj5 and Mj6 being in the first one, and Mj2, Mj4 and Mj3 in the second one. The current data complement the studies in the area of genetic resources, therefore they could be included in conservation programs for protection of rare and endangered plant species.

Keywords: Moehringia jankae; genetic diversity; inter-simple sequence; (ISSR) markers

Introduction

M. jankae is a Balkan endemic representative of *Moehringia* genus (Linnaeus, 1753), part of *Alsinoideae* subfamily of *Caryophyllaceae* family. *Moehringia* genus is distributed in the moderate zone of the Northern hemisphere with most species occurring in Europe. Due to the specifics of their habitats (mainly rock crevices) the species are basically local or regional endemics (Fior et al., 2006; Fior & Karis, 2007; Loritea et al., 2018). In Bulgaria populations of *M. jankae* have been found in the North-Eastern part (Shumen region) and Eastern Balkan Range - Sinite Kamani Natural Park (Sliven) (Delipavlov & Cheshmedzhiev, 2003; Grozeva et al., 2004, 2016; Assyov & Petrova, 2012; Zahariev, 2014; Stoeva, 2015). The status of the species is endangered, therefore it is protected by the Biological Diversity Act, under Annex I of the Bern Convention and according to the criteria of the IUCN Red Lists of Threatened Plants. On a global scale, *M. jankae* has been rated as "Data Deficient (DD)". The species is included in Annex II of Directive 92/43/EEC and in the Red Book of Bulgaria, vol. 1. Plants and fungi, under the category" endangered".

Over the last few years, the populations of *M. jankae* have been identified only on the territory of Sinite Kamani Natural Park in Sliven, Bulgaria (Grozeva et al., 2016; Zhelyazkova et al., 2018). These populations described vary greatly in area and size from 1.2 to 796 m^2 and from 8 to 73 specimens on quartz porphyry rock formations and conglomerates. Lichens, mosses and *M. grisebachii* have been identified as accompanying conservation significant species.

The survival of endangered plants is directly linked to the conservation of their genetic diversity by maintaining viable populations. Due to the habitats preferred by *M. jankae* (rock crevices), the species exists in small and isolated populations and/or in low-density populations. This isolation is both a refuge because of its difficult accessibility (Polunin, 1980; Thompson, 2005) and a threat (Rogstad & Pelikan**,** 2013). Small populations are particularly susceptible to inbreeding depression and genostasis, and hence reducing the plasticity of specimens that had lost their genetic variability also loses the potential of adaptive responses (Frankham et al., 2010). Monitoring, knowledge and maintenance of genetic diversity in endangered species is a major factor in their preservation and conservation (Frankham et al., 2010; Allendorf et al., 2012; Rogstad & Pelikan, 2013). Therefore, the aim of this study was to evaluate the genetic diversity of 11 natural populations of *M. jankae*

Fig. 1. *Moehringia jankae*

Material and Methods

Samples collection

During the vegetation period of 2017–2019 plants from a total of 11 natural populations of the *M. jankae* species (Fig. 1) were collected, localized in various regions, according to the data reported by Zhelyazkova et al. (2018) and to the species distribution in Bulgaria (Table 1). Following identification based on their morphological characteristics, plants were placed in silica gel and stored at -18°C for subsequent DNA extraction and PCR amplification.

DNA extraction and PCR amplification

Genomic DNA was extracted via Plant DNA Preparation Kit (Jena Bioscience) by optimizing the original protocol of the manufacturer. DNA quality and yield have been established by Nano Vue Plus spectrophotometer and Agarose gel (1%) electrophoresis, visualized on Transilluminator (Bio-Imaging System). DNA samples with purity from 1.6 – 1.9 (260/280 nm) were used for preparation of working solu-

Table 1. Distribution of studied populations of *M. jankae*

Pop	Location	Longitude, Latitude and Altitude	m^2/N specimen
Mj1	The rock formation in Kamilata area	N 42° 42.603′, E 26° 22.180′, 857 m	4,8 /33
Mj2	Along Haiduschka pateka area east of hotel complex Karandila	N 42° 42.704′, E 26° 22.261′, 889 m	82/33
Mj3	Micro dam area	N 42° 42.790′, E 26° 22.612′, 972 m	26 / 22
Mj4	350 m. south of hotel complex Karandila	N 42° 42.709′, E 26° 22.355′, 933 m	2,5 / 14
Mj5	450 m. southwest of hotel complex Karandila	N 42° 42.712′, E 26° 22.252′, 908 m	55,5/61
Mj6	The rocks south-east of Kamilata area	N 42° 42.593′, E 26° 22.196′, 851 m	4/5
Mj7	The rocks between hotel complex Karandila and Kamilata area	N 42° 42.726′, E 26° 22.349, 952 m	1,5 /8
Mj8	The north of Kamilata area	N 42° 42.673′, E 26° 22.217′, 866 m	70 /13
Mj9	The rock formations near Haiduschka pateka area	N 42° 42.881′, E 26° 22.458′, 620 m	22 / 73
Mj10	The east of Kamilata area	N 42° 42.647′, E 26° 22.198′, 869 m	38 / 15
Mj11	Kaloyanovi kuli area	N 42° 42.755′, E 26° 23.015′, 756 m	19 / 45

tions with concentration 100 ng/µl and stored at 4ºC for PCR amplification. The PCR amplifications were carried out in a total volume of 25 µl, containing 1 µl genomic DNA, 12.5 µl Red Taq DNA Polymerase 2×Master Mix, 1.5 µl Primer (Bioneer) and 10 µl nuclease free ddH_2O (Sigma). Amplification was performed on Doppio Gradient 2 × 48 well thermal cycler (VWR®, Germany), following a protocol by Pourhosseini (2018): an initial denaturation at 94ºC/5 min, followed by 35 cycles at 94°C/45 s denaturation, 49 – 56.8°C specific annealing temperature (Table 2) for 1 min, extension at 72ºC/1 min; and final extension at 72°C/8 min. PCR was optimized by modification of annealing temperature to specific annealing temperature ($sT_a°$) until maximum results were reached with each separate primer. The recording / identification of ISSR-PCR amplified products was performed through the horizontal electrophoresis, on 1.5% agarose gel with 1×TBE buffer for 50 min at 80 V/cm and visualized on Electrophoresis Gel Imaging Analysis System (Bio-Imaging Systems, Israel). Gels were stained with fluorescent nucleic acid dye GelRed® (Biotium, USA) 7 µl of product applied mixed with 1.5 µl loading buffer and 100$^+$ DNA-ladder (100 – 3000 bp). The presence (1) and absence (0) of bands were used for binary character matrix. Unclear bands were noted as missing value.

Statistical analysis

Efficiency of selected markers was determined by calculating the following parameters: polymorphic information content (PIC) (Botstein et al., 1980; Roldan-Ruiz et al., 2000), effective multiplex ratio (EMR) (Powell et al., 1996; Nagaraju et al., 2001), marker index (MI) (Varshney et al., 2007) and resolving power (RP) (Prevost & Wilkinson 1999).

SPSS for Windows was used for performing Hierarchical Cluster analysis based on Euclidean genetic distance, which illustrated amount of genetic diversity in 11 studied populations. Principal Component Analysis also divides populations based on their genetic diversity. GenAlEx (Peakall & Smouse, 2006) ver. 6.5 Nei's (1973; 1978); Nei & Roychoudhury (1974) was used both for the parameters: different number of alleles (Na), effective number of alleles (Ne), Shannon's Information Index (I) (Lewontin, 1972), expected (He) and unbiased (uHe) expected heterozygosity, percentage of polymorphic bands (PPB) and for Principal coordinate analysis (PCoA) with the data from Pairwise Population Matrix of Nei Unbiased Genetic Distance. Inter- and intra-population variability was also evaluated by Analysis of Molecular Variance (AMOVA) in this statistical package. The data binary matrix of 5 ISSR markers of 11 populations of *M. jankae* was converted into genetic similarity matrix using Jaccard coefficient (Jaccard, 1908).

Results

In the present study, a total of 59 polymorphic loci were identified through the amplification with 5 selected ISSR primers, varying in range from 180 to 2000 bp., with their number from 3 to 20 at (ATC)6 and (AG)8YC. The percentage of polymorphic bands (PB) is from 50-95.24%, respectively. EMR varied from 3.48-5.10 for (AG)8C and (GACA)4, averaging 4.42. The mean value of PIC for the primers was 0.64, with the highest value (0.80) at (AG)8C - and the lowest one (0.40) at primer (ATC)6 . Resolving power (Rp) was calculated within the range from 11.32 for (ATC)6 to 37.85 for (AG)8C, averaging 27.66. Marker index (MI) has the highest value for (AG)8YC -3.57 and the lowest 1.52 for (ATC)6 (Table 2). The values obtained for PIC, Rp and MI pointed that primers (AG)8C and (AG)8YC are more informative than the others. On the other hand, results according to effective multiplex ratio (EMR) demonstrated that (AG)8YC and (GACA)4 were the most efficient "marker-primer" system , in accordance with Chesnokov & Artemyeva, 2015. The high values (0.64) obtained for polymorphic information content (PIC) were in correlation with high and medium polymorphism according to the polymorphism rate scale, as follows: PIC < 0.25 – low; 0.25÷0.5 – medium and

Table 2. ISSR primers used in assessment genetic diversity of 11 natural populations of *M. jankae* and their parameters: specific annealing temperature ($sT_a°$), total bands (TB), polymorphic bands (PB), monomorphic bands (MB), effective multiplex ratio (EMR), polymorphic information content (PIC), resolving power (Rp), Marker index (MI)

Primer	Sequence	$sT_a°$	TB	PB	MB	% PB	EMR	PIC	Rp	MI
(AG)8C	AGAGAGAGAGAGAGAGC	52.3	13	12	1	92.31	3.48	0.80	37.85	2.80
(AG)8YC	AGAGAGAGAGAGAGAGYC	55	21	20	1	95.24	5.10	0.70	36.64	3.57
(ATC)6	ATCATCATCATCATCATC	49	6	3	3	50	3.83	0.40	11.32	1.52
(GACA)4	GACAGACAGACAGACA	52	13	11	2	84.62	5.01	0.63	23.35	3.16
(CA)8RG	CACACACACACACACARG	56.8	15	13	2	86.67	4.71	0.66	29.15	3.13
Total			68	59	9					
	Mean						4.42	0.64	27.66	2.84

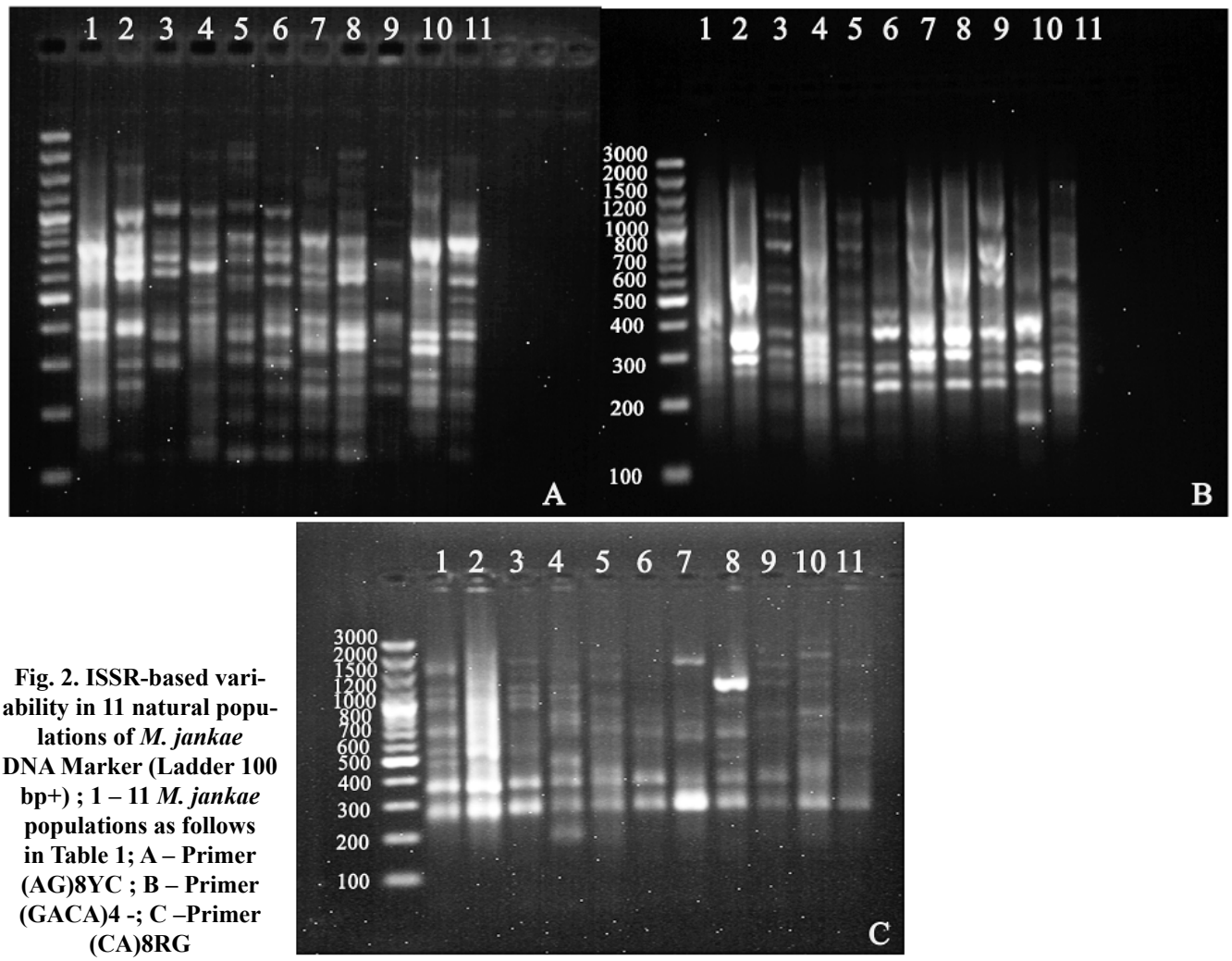

Fig. 2. ISSR-based variability in 11 natural populations of *M. jankae* DNA Marker (Ladder 100 bp+) ; 1 – 11 *M. jankae* populations as follows in Table 1; A – Primer (AG)8YC ; B – Primer (GACA)4 -; C –Primer (CA)8RG

Table 3. Distribution of the bands obtained in studied populations

Population	Mj1	Mj2	Mj3	Mj4	Mj5	Mj6	Mj7	Mj8	Mj9	Mj10	Mj11
Bands	13	9	21	18	17	6	11	11	15	14	13
Bands frequency ≥ 5%	13	9	21	18	17	6	11	11	15	14	13
Private (rare) bands	1	0	0	0	1	0	0	0	1	0	0
Locally common bands (frequency ≤25%)	0	0	1	1	0	0	0	0	0	1	1
Locally common bands (frequency ≤50%)	3	2	8	7	4	1	0	0	2	4	4

> 0.5 – high polymorphism (Xie et al., 2010). Fig. 2 represents ISSR-based variability based on primers (AG)8YC, (GACA)4 and (CA)8RG.

The most numerous bands were noticed in populations Mj3 (21) and Mj4 (18), with frequency between 25 and 50% and the least numerous ones were identified in Mj6 (6) and Mj3 (9). The lowest was the frequency of bands in Mj 7 and Mj 8 (from 5 to 25%). The mean value for expected heterozygosity was the highest in Mj3 and Mj4, and the lowest for populations Mj6 and Mj2. Specific inter-simple sequence repeat band was noticed in population 1 (900 bp) with primer (AG)8YC, in population 5 (450 bp) with primer (AG)8C and in population 9 (180 bp) with primer (AG)8C (Table 3, 4, Fig. 3).

It has been found for the *M. jankae* populations that Na varies from 0.33 (Mj6) to 1.56 (Mj3) with a total mean

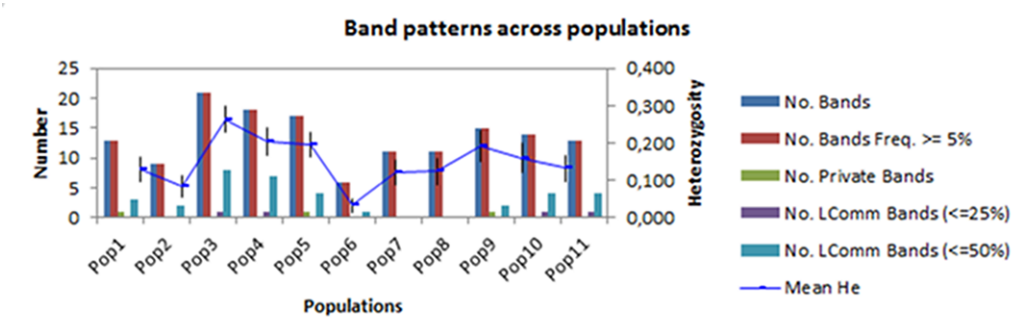

Fig. 3. Illustration of band frequency and mean expected heterozygotes

Table 4. Genetic diversity of 11 natural populations of *M. jankae* based on 5 ISSR markers, mean and standard deviations of different (Na) and effective (Ne) number alleles, Shannon's Information Index (I), expected (He) and unbiased expected heterozygosity (uHe) for data and assuming Hardy-Weinberg Equilibrium(HWE), percentage of polymorphic bands (PPB).

Population	Na	Ne	I	He	uHe	PPB%
Mj1	0.93 (0.192)*	1.20 (0.055)	0.204(0.048)	0.129(0.032)	0.144(0.035)	44.4
Mj2	0.63 (0.178)	1.14 (0.053)	0.133(0.043)	0.085(0.029)	0.094(0.032)	29.6
Mj3	1.56 (0.163)	1.45 (0.072)	0.398(0.050)	0.263(0.036)	0.292(0.040)	77.8
Mj4	1.26 (0.182)	1.34 (0.070)	0.307(0.054)	0.203(0.038)	0.226(0.042)	59.3
Mj5	1.22 (0.187)	1.31 (0.059)	0.301(0.052)	0.196(0.035)	0.218(0.039)	59.2
Mj6	0.33 (0.131)	1.05 (0.028)	0.052(0.029)	0.033(0.019)	0.037(0.021)	11.1
Mj7	0.78 (0.187)	1.19 (0.057)	0.186(0.050)	0.121(0.033)	0.135(0.037)	37.0
Mj8	0.74 (0.182)	1.21 (0.063)	0.185(0.053)	0.124(0.036)	0.138(0.040)	33.3
Mj9	1.07 (0.192)	1.34 (0.08)	0.283(0.058)	0.191(0.041)	0.212(0.046)	51.9
Mj10	0.95 (0.184)	1.28 (0.072)	0.235(0.057)	0.160(0.039)	0.178(0.044)	40.7
Mj11	0.90 (0.187)	1.22 (0.061)	0.203(0.051)	0.132(0.034)	0.147(0.038)	40.8
Total	0.94(0.056)	1.25(0.019)	0.226(0.016)	0.149(0.011)	0.165(0.012)	44.11

* in parentheses are labeled values of standart deviation

of 0.94, while values of Ne were from 1.05 (Mj6) to 1.45 (Mj3) with a total mean of 1.25. I, He and uHe in the studied populations showed mean values 0.226, 0.149 and 0.165, respectively. The values distinguish the population Mj3 (I = 0.398; He = 0.263; uHe = 0.292) and Mj4 (I = 0.307; He = 0.203; uHe = 0.226) with the highest intra-population genetic diversity and population Mj6 (I = 0.052; He = 0.033; uHe = 0.037) and with the lowest one in Mj2 (I = 0.133; He = 0.085; uHe = 0.094), respectively. The level of genetic diversity calculated as percentage of polymorphic bands in Mj4 (59.3%) was higher than the others and lower in Mj6 population (Table 4).

The Analysis of molecular variance (AMOVA) (Table 5, Fig. 4) confirms the data from parameters: Na, Ne, I, He, uHe, and PPB, showing a significant level of intra-population diversity in the studied populations of *M. jankae*, with a p-value (< 0.001), pointed statistical significance and the amount for F_{st} = 0,276 (AMOVA – derived among population variability (Wright, 1951, 1965, 1978), where F_{st} is Est.Var.Among population / (Est.Var.Within population + Est.Var.Among population).

Table 5. Data of AMOVA analysis base on 5 ISSR markers in 11 studied population of *M. jankae*

Source of variation	df	SS	MS	Est. Var.	% variation
Among the populations	10	87.055	8.705	1.141	28
Within the populations	44	132.000	3.000	3.000	72
Total	54	219.055	11.705	4.141	100

*df – degree of freedom
SS – total sum of square
MS – midle square
Est. Var. – estimated variance

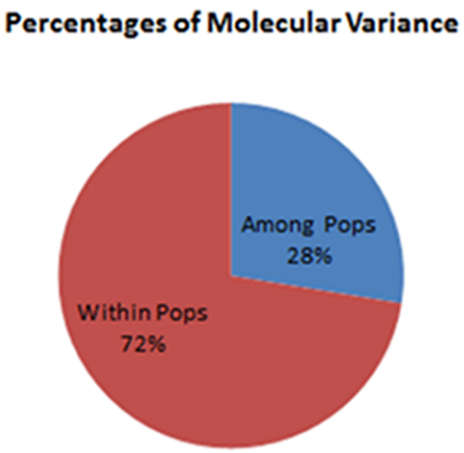

Fig. 4. Illustration of AMOVA results for genetic diversity of 11 populations of *M. jankae*

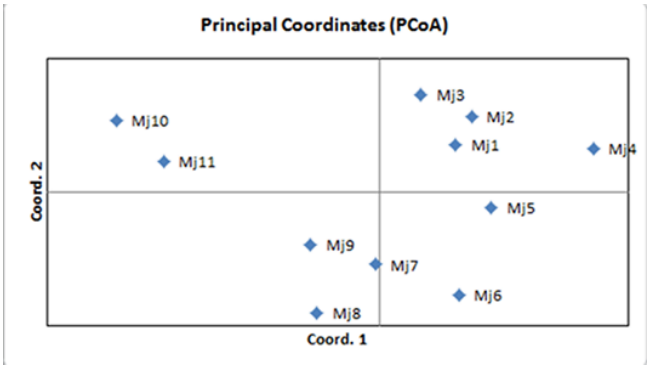

Fig. 5. Two-dimensional plot of the principal coordinate analysis (PCoA) of 11 natural populations of *M. jankae* based on 5 ISSR markers

Pairwise Nei's (1978) genetic distances (GD) were used for principal coordinate analysis PCoA analysis (Table 6, Fig. 5). The percentage of variation explained by the first 3 axes was 47.32, 20.15 and 18.23, accounting for 85.07% of the genetic similarity variance. GD values varied from 0.006 (Mj10 - Mj11) to 0.266 (Mj4 - Mj10), which also indicated for a relatively low level of genetic diversity among the populations of *M. jankae*.

Principal Component Analysis (PCA) (Fig. 6) with binary data obtained from the used ISSR markers, extracted 2 components with % Variance 49.220 and 9.394, and Cumulative % 49.220 and 58.614. Based on Component Matrix, populations of *M. Jankae* were divided into two groups. The first one comprises populations Mj2, Mj3, Mj4, Mj5 and Mj6, while the second one – populations Mj1, Mj7, Mj8, Mj9, Mj10 and Mj11 (Table 7).

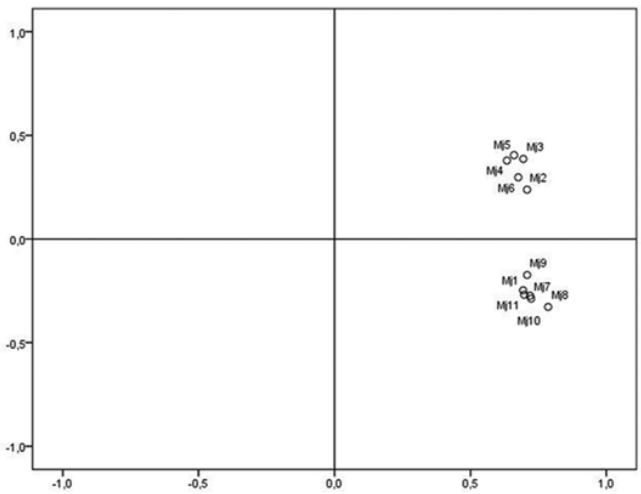

Fig. 6. PCA genetic diversity of 11 natural populations of *M. jankae* based on ISSR study

Table 6. Pairwise Population Matrix of Nei Unbiased Genetic Distance (above diagonal) and Genetic Identity (below diagonal)

	Mj1	Mj2	Mj3	Mj4	Mj5	Mj6	Mj7	Mj8	Mj9	Mj10	Mj11
Mj1	***	0.988	0.943	0.908	0.992	0.992	0.948	0.905	0.919	0.861	0.896
Mj2	0.012	***	0.944	0.886	0.979	0.899	0.930	0.873	0.901	0.830	0.883
Mj3	0.058	0.058	***	0.967	0.969	0.888	0.954	0.909	0.950	0.893	0.920
Mj4	0.097	0.121	0.033	***	0.955	0.882	0.910	0.850	0.897	0.767	0.789
Mj5	0.008	0.021	0.031	0.046	***	0.965	0.985	0.940	0.957	0.845	0.892
Mj6	0.081	0.106	0.119	0.126	0.035	***	0.976	0.895	0.899	0.821	0.862
Mj7	0.054	0.072	0.047	0.094	0.015	0.024	***	0.971	0.983	0.896	0.944
Mj8	0.100	0.135	0.095	0.162	0.062	0.111	0.029	***	0.986	0.871	0.918
Mj9	0.085	0.104	0.051	0.109	0.044	0.107	0.017	0.014	***	0.919	0.951
Mj10	0.150	0.187	0.113	0.266	0.168	0.198	0.109	0.138	0.085	***	0.994
Mj11	0.110	0.125	0.083	0.237	0.114	0.148	0.057	0.086	0.050	0.006	***

Table 7. Component matrix with correlation among 11 studied population of *M. jankae* obtained with Principal Component Analysis (PCA) on based ISSR data

population	Matrix components	
	1	2
Mj1	0.694	-0.248
Mj2	0.709	0.239
Mj3	0.695	0.387
Mj4	0.635	0.379
Mj5	0.661	0.405
Mj6	0.676	0.298
Mj7	0.724	-0.288
Mj8	0.786	-0.328
Mj9	0.709	-0.174
Mj10	0.720	-0.274
Mj11	0.698	-0.270

Hierarchical Cluster based on Euclidean genetic distance sums up and confirmed the results obtained from PcoA and PCA analyses. Identically with Principal Component Analysis, the cluster analysis (Fig. 7) divided studied populations into 2 main clusters. Cluster A comprised Mj7, Mj8 and Mj10 in the first subcluster, Mj1, Mj11 and Mj9 in the second subcluster. The highest degree of genetic similarity was established between the populations Mj7 and Mj8. Cluster B consisted of 2 subclusters, with Mj5 and Mj6 being in the first one, and Mj2, Mj4 and Mj3 in the second one. In cluster **B**, the highest genetic similarity the observed is within the populations Mj5 and Mj6. The level of genetic identity among the different populations has been summed up and confirmed by Jaccard's (1908) (Table 8).

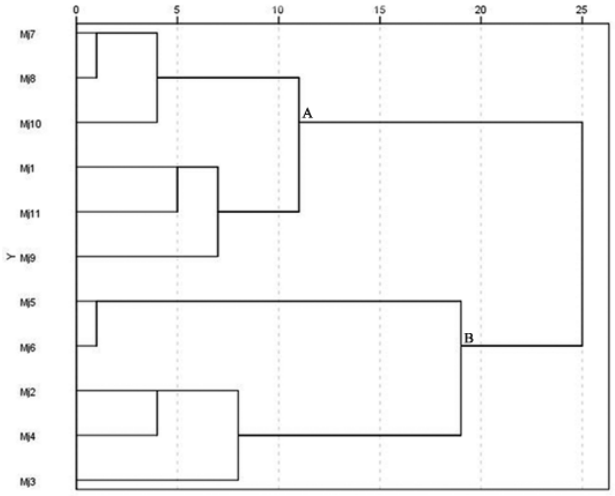

Fig. 7. Dendrogram of Genetic diversity of 11 natural studied populations

Discussion

The results from the present study showed relatively high level of genetic identity between the *M. jankae* populations 0.767-0.994 (Nei Unbiased Genetic Identity (Nei, 1978)) and 0.362-0.615 (Jaccard similarity (Jaccard, 1908)), at the expense of the intra-population variation (72% of molecular variance). In similar studies in the area of plant phylogenetics, this is accounted for by the area of distribution of the individual species. Species with wide area, such as *Silene latifolia* and *C. rotudifolia* show lower values of similarity value 0.28-0.61 and 0.27-0.83, respectively higher level of genetic diversity (Whitty et al., 1994; Vellekoop et al., 1996), while the rare and endemic species restricted within their area have a significantly higher similarity value, as established for *Limonium cavanillesii* (similarity value = 1) and *Adenophora grandiflora* (similarity value = 0.99) (Yoo et al., 1996; Palacios & Gonzalez-

Table 8. Genetic similarity matrix of 11 studied population of *M. jankae* by Jaccard (1908) based on 5 ISSR markers

	Mj1	Mj2	Mj3	Mj4	Mj5	Mj6	Mj7	Mj8	Mj9	Mj10	Mj11
Mj1	1										
Mj2	0.489	1									
Mj3	0.478	0.574	1								
Mj4	0.392	0.604	0.500	1							
Mj5	0.370	0.380	0.558	0.429	1						
Mj6	0.413	0.479	0.438	0.440	0.600	1					
Mj7	0.395	0.404	0.362	0.396	0.475	0.452	1				
Mj8	0.523	0.553	0.479	0.451	0.435	0.417	0.615	1			
Mj9	0.475	0.444	0.432	0.435	0.381	0.395	0.486	0.550	1		
Mj10	0.523	0.460	0.449	0.451	0.404	0.478	0.575	0.556	0.409	1	
Mj11	0.535	0.440	0.458	0.404	0.413	0.457	0.442	0.568	0.452	0.500	1

Candelas, 1997) and respectively significantly weaker level of genetic diversity (Mayol & Rosselló, 2001; Sheeja et al., 2009; Verma et al., 2009; Hua-hui et al., 2011). Similar results have been reported by Zheng et al. (2001) studying the genetic diversity of endemic and endangered species *Anisodus tanguticus* (Solanaceae). Authors reported 0.776 – 0.941 for Nei's genetic identity and 67.02% intra-population diversity at the expense of 32.98% inter-population on 10 populations of the species, examined with RAPD primers . Both these results and the ones achieved in the present study are comparable to Nybom (2004) and Nybom & Bartish (2000). Within the DNA-based studies Φ_{ST} gives almost identical information about ISSR and RAPD markers (Nybom, 2004). Concerning the connection between territorial distribution and genetic diversity, populations *Anisodus tanguticus* form 2 subclusters, which according to Zheng et al. (2001) are due to the complex topography of the region, including high mountains that could hinder the gene flow and thus stimulate intra-population differentiation. A total of 11 studied populations of *M. jankae* were allotted to 2 subclusters as well, confirmed by the applied PCA analysis. To one of the subclusters populations with the highest values for I, He, uHe Mj3 (0.398; 0.263; 0.292), Mj4 (0.307; 0.203; 0.226) and Mj5 (0.301; 0.196; 0.218) were seen to belong and along with Mj2 they form their cluster around the area of their distribution – Microyazovir and Karandila areas. As an exception to that group are the data about Mj6 with the highest recorded indices of the monitored values I = 0.052; He = 0.033, uHe = 0.037. This could be accounted for both by its location isolated by rocky massifs (the rocks south-east of Kamilata area), and the small area and number of this population (4 m^2/5 specimens). The other group based on our results is formed mainly around Kamilata area Mj1 (The rock formation in Kamilata area), Mj7 (The rocks between hotel complex Karandila and Kamilata area), Mj8 (The north of Kamilata area) Mj10 (The east of Kamilata area). The presence of both specific bands in Mj1, Mj5 and Mj9 and bands of frequency less than 25% in Mj3, Mj4, Mj10 and Mj11 could be accounted for by decreasing the area and number of populations, which increases the level of genetic differentiation between single isolated populations, in accordance with Ellstand & Elam (1993).

Genetic diversity among populations and specimens has been studied in 2 other representatives of Moehringia genus - *M. lebrunii* and *M. Sedoides*, spreated in Maritime Alps, based on ISSR markers by Munito et al. (2006). The degree of genetic variability between the populations represented as $G_{st,}$ similar to F_{ST} (Liang et al., 2015) in the populations of *M. Sedoides* was 0.355 and 0.255 for the populations of *M. lebrunii*, with *M. Sedoides* having, respectively, good ecological adaptability although it grows only on lime rocks (Munito et al., 2006). In spite of that changes in the ecological and historical models are the probable reason both for reduction of the populations and their fragmentation, on which their genetic diversity is based.

Conclusions

On the basis of the results obtained in this study, we found higher intrapopulation diversity than interpopulation one. The level of genetic diversity in *M. jankae* is closely related to the resistance of the species to long-term biotic and abiotic changes in the environment. Inter-simple sequence repeat (ISSR) is successfully applicable for studying the genetic structure and diversity of plant species. In our study, the efficiency of ISSR to detect polymorphisms in 11 populations of *M. jankae* reaches 95.24% PB, which was proven by the reported high values of the parameters characterizing each individual primer.

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