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Genetic and chemical diversity among *Salvia multicaulis* populations employing AFLP markers and the essential oil profile

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Abstract

Salvia multicaulis is a widespread medicinal plant growing in north, northwest, and center of Iran. In this study, the aerial parts of 29 specimens belonging to five geographical populations were collected and dried at full flowering stage. AFLP molecular markers were applied for genetic diversity analysis. Essential oil was extracted by hydro-distillation and its chemical compositions were analyzed by GC/MS. AFLP results led to the recognition of two different genotypes. The results demonstrated high molecular affinity among Azerbaijan and Kurdistan populations. However, Tehran showed much more correlations with Isfahan and Kohgiluyeh-va-Boyerahmad populations. The AMOVA test results, demonstrated high within population genetic variability. STRUCTURE analysis revealed some degree of population genetic fragmentation that was principally due to genetic difference occurred between Azerbaijan and the rest of the studied populations. Overall, 40 constituents were determined among which, α-pinene (11.2–19.9%) and 1,8-cineole (10.2–20.4%) were distinguished as two major compounds. Tehran showed closer affinity to Isfahan and Kohgiluyeh-va-Boyerahmad. The results of molecular studies were in accordance with those obtained by the chemical compositions. Therefore, ecological factors and geographical distance as well as genetics play an important role in the species evolution and distribution in different parts of the country.

Keywords: Chemical polymorphism, GC/MS analysis, *Lamiaceae*, molecular affinity, molecular markers

تنوع ژنتیکی و شیمیایی در جمعیت‌های *Salvia multicaulis* با استفاده از مارکرهای AFLP و ویژگی‌های اسانس*

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خلاصه

Salvia multicaulis (نعناییان) گیاهی دارویی با پراکنش وسیع در شمال، شمال‌غرب و مرکز ایران است. در این مطالعه، بخش‌های هوایی ۲۹ نمونه مربوط به پنج جمعیت جغرافیایی جمع‌آوری و در مرحله گل‌دهی کامل خشک شدند. مارکرهای مولکولی AFLP برای آنالیز تنوع ژنتیکی به کار برده شد. اسانس به وسیله تقطیر آبی استخراج و ترکیبات شیمیایی آن توسط آنالیز GC/MS شناسایی شدند. نتایج AFLP منجر به شناسایی دو ژنوتیپ متفاوت گردید. نتایج شباهت مولکولی بالایی بین جمعیت‌های آذربایجان و کردستان را نشان داد. با این حال، جمعیت تهران تشابه بیشتری به جمعیت‌های اصفهان و کهگیلویه و بویراحمد نشان داد. نتایج تست AMOVA تنوع بین جمعیتی بالایی را نشان داد. آنالیز STRUCTURE، درجاتی از قطعه قطعه شدن ژنتیکی جمعیت را آشکار ساخت که به طور کلی، به دلیل تفاوت ژنتیکی رخ داده بین جمعیت آذربایجان و سایر جمعیت‌های مورد مطالعه بود. در مجموع، ۴۰ ترکیب شناسایی شد که در میان آن، آلفا-پینن (۱۹/۹-۱۱/۲٪) و ۱ و ۸-سینئول (۲۰/۴-۱۰/۲٪) به عنوان دو ترکیب اصلی تشخیص داده شدند. تهران بیشترین شباهت را به اصفهان و کهگیلویه و بویراحمد نشان داد. نتایج مطالعات مولکولی با نتایج به دست آمده از ترکیبات شیمیایی مطابقت داشت. بنابراین، فاکتورهای بوم‌شناختی و فاصله جغرافیایی و نیز ژنتیک نقش مهمی را در تکامل و انتشار گونه در نقاط مختلف کشور ایفا می‌کنند.

واژه‌های کلیدی: آنالیز GC/MS، پلی‌مورفیسم شیمیایی، تشابه مولکولی، مارکرهای مولکولی، نعناییان

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Introduction

Salvia L. (*Lamiaceae*) considered an important as well as the largest medicinal plant within the family with almost 1000 species worldwide that grows in temperate and warm environments (Davis 1982, Mohammadhoseini *et al.* 2008). Iran with almost 60 native species is regarded as one of its diversification centers (Hedge 1982, Walker *et al.* 2004, Jamzad 2012, Kharazian 2014). *Salvia multicaulis* Vahl. is an aromatic perennial herbaceous species widely distributed in numerous parts of the Middle Eastern countries including north-west, west, and center of Iran, Turkey, Lebanon and Iraq (Jamzad 2012, Talebi *et al.* 2021). It has some pharmaceutical properties like other species nested in the genus including antimicrobial, anti-inflammatory, and pain-relieving effects (Asadi-Samani *et al.* 2019). Since, AFLP (amplified fragment length polymorphism) as a DNA-based molecular marker, has high reproducibility and its simultaneous capability to distinguish polymorphisms by employing a single assay is accepted by several scientists, it is applied for assessment of genetic diversity and population structure of various plants including *Salvia* species (Wang *et al.* 2007, Zhang *et al.* 2009, Qiao *et al.* 2020). Recently, genetic structure related to ten Iranian populations of *S. multicaulis* has been reported employing ISSR molecular markers (Talebi *et al.* 2021). Moreover, essential oil chemical composition and antibacterial effects of this species have also been reported by several authors (Senatore *et al.* 2004, Mohammadhoseini *et al.* 2008, Asadi-Samani *et al.* 2019, Talebi *et al.* 2021). Nevertheless, some of the studies have been focused on the essential oil constituents among different populations of the species (Ulubelen *et al.* 1997, Fahed *et al.* 2016, Talebi *et al.* 2021).

According to the latest studies on plant morphology, several variations in some morphological traits like bract, calyx, and basal leaf petiole size, have been recognized among the studied populations in different ecological conditions and, therefore, occurrence of different infra-specific taxonomic levels in Iran could be expectable (Talebi *et al.* 2017). Regarding the mentioned above statements, the present study aimed to investigate the capability of AFLP molecular markers to determine the level of diversity among specimens collected from five different localities of Iran for the first time, and also defining probable variations in the essential oil profiles among the studied populations.

Materials and Methods

- Plant materials and DNA extraction

The aerial parts of 29 individuals related to five geographical populations of *Salvia multicaulis* from five different provinces of Iran i.e., Tehran, Isfahan, Kohgiluyeh-va-Boyerahmad, Azerbaijan, and Kurdistan, were collected during May 2015. Dried materials (by silica gel) at full flowering stage were applied for further analyses. Six specimens were sampled in each population (except for Tehran population with five specimens). The voucher specimen of each population is deposited at IAUH (Identified by Dr. I. Mehregan). Details of sampling sites are represented in table 1 and figure 1.

Total genomic DNA was then extracted from the dried leaves employing the DNeasy Plant DNA Extraction kit (Qiagen). The quality of extracted DNA was assessed on 1% agarose gel electrophoresis and its concentration was also measured by Nano-Drop™ spectrophotometer (Thermo Fisher Scientific, USA).

- AFLP markers

The AFLP molecular markers were conducted based on the previous published protocols with negligible alterations (Vos *et al.* 1995). Concisely, the extracted DNA was digested using EcoRI and MseI adaptors as restrictive enzymes, and then, the adaptors were ligated by T4 DNA ligase (Boehringer Mannheim) to the digested DNA fragments. Pre selective PCR amplification was performed employing the following program profile: 72 °C for 2 min., followed by 20 cycles of 20 sec. at 94 °C; 30 sec. at 56 °C and 2 min. at 72 °C and a final extension step of 30 min. at 60 °C. Selective amplification was also accomplished by three pairs of primers. The names and sequences of applied primers are listed in table 2. The following cycle profile was applied for selective PCR amplification: 2 min. at 94 °C, 10 cycles: 20 sec. at 94 °C, 30 sec. at 56–65 °C, 2 min. at 72 °C, and 25 cycles: 20 sec. at 94 °C, 30 sec. at 56 °C, 2 min. at 72 °C. All amplifications were done in a BioRad thermocycler (BioRad Laboratories Inc., Hercules, CA, USA). The amplified DNA fragments were then isolated and visualized by running on 1% agarose gel and stained by gel red. DNA ladder was also applied for evaluation of the amplicon size. Purified PCR products were then sequenced by ABI 3730 xl sequencer (Thermo Fisher Scientific Applied BiosystemsTM) and attained chromatograms were edited employing Genemarker software (SoftGenetic LLC, USA).

Table 1. List of five geographical populations of *Salvia multicaulis* from five provinces accompanied by their related data

Population and specimen No.	Locality (province)	Longitude	Latitude	Altitude (m)	Herbarium No.
1 (1–6)	Kurdistan	46°25'	35°30'	1720	IAUH-000014495
2 (7–12)	W. Azerbaijan	46°12'	36°31'	1500	IAUH-000014496
3 (13–18)	Isfahan	50°40'	33°59'	1950	IAUH-000014497
4 (19–23)	Tehran	51°00'	35°48'	2020	IAUH-000014498
5 (24–29)	Kohgiluyeh-va-Boyerahmad	51°31'	30°31'	1800	IAUH-000014499

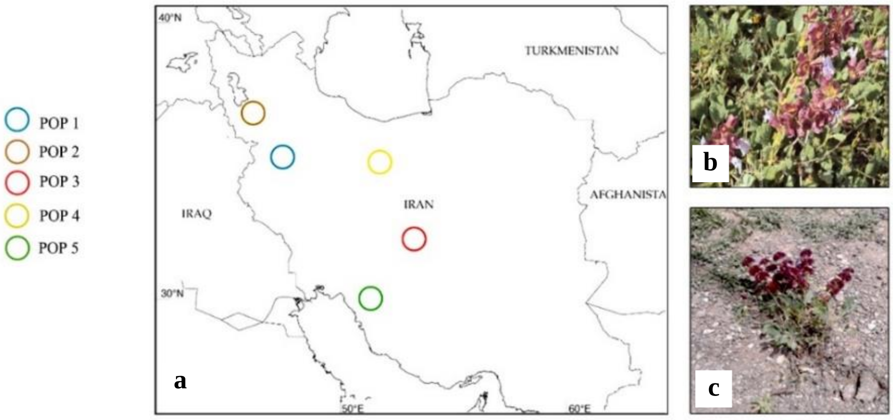

Fig. 1. The distribution, natural habitat, and morphology of *Salvia multicaulis*: a. Distribution map of the five local populations of *Salvia multicaulis* in Iran (Pop 1: Kurdistan, Pop 2: Azerbaijan, Pop 3: Isfahan, Pop 4: Tehran, and Pop 5: Kohgiluyeh-o-Boyerahmad populations. Note: Populations of different localities are also differentiated with different colors), b & c. Plant and its natural habitat (Photograph from Taleghan, Tehran province).

Table 2. Primer names and sequences used in selective PCR amplification (Note: The AFLP primer names are abbreviated based on the standard nomenclature of AFLPs proposed by KeyGene (https://wheat.pw.usda.gov/ggpages/keygeneAFLPs.html)

Forward primer	Sequence	Reverse primer	Sequence
E-38	5’HEX-GACTGCGTACCAATTCACT-3’	M-57	5’-GATGAGTCCTGAGTAACGG-3’
E-45	5’FAM-GACTGCGTACCAATTCATG-3’	M-54	5’-GATGAGTCCTGAGTAACCT-3’
E-40	5’NED-GACTGCGTACCAATTCAGC-3’	M-55	5’-GATGAGTCCTGAGTAACGA-3’

- AFLP molecular analyses

For evaluation of population genetic diversity, the AFLP molecular data were entered into a binary matrix representing the AFLP profile of each sample. Then, the results were analyzed using GenAlEx 6.5 in Excel software (Peakall *et al.* 2006, 2012). The genetic structure of *S. multicaulis* populations was studied by Bayesian based model STRUCTURE 2.3.4 software (Pritchard *et al.* 2000). AMOVA assay with 1000 permutations was accomplished using GenAlex 6.4 (Peakall & Smouse 2006).

- Essential oil extraction and GC/MS analysis

To obtain essential oil, 100g of an air-dried powder related to the aerial parts of the wild sample of *S. multicaulis* from each isolated population was applied for hydro-distillation in three hours employing a Clevenger-type apparatus (Clevenger 1928). The essential oil constituents were analyzed by GC/MS. The analytical GC/MS system applied in this study was an Agilent 7890A/5975C GC/MS system, equipped with a DB-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm; ISO15303). The column temperature programming was as follows: The initial temperature at 60 °C immediately increased to 220 °C at a rate of 3 °C/min., and then the temperature increased to 260 °C at 20 °C/min. and held at this temperature for 5 min. The injection volume was 2 µL and the injector temperature was 260 °C. Helium was applied as a carrier gas at a linear velocity of 30.6 cm/s; split ratio of 1:100, ionization energy of 70 eV, scan time 1s, and scan range of 40–300 amu. The transfer line was heated at 280 °C.

Results

- Genetic diversity and population genetics

In the present study, genetic variations of five different populations belonging to *Salvia multicaulis* species was assessed by AFLP molecular markers for the first time in Iran (Table 1, Fig. 1). Two different genotypes were determined in the mentioned populations [Genotypes A (green) and B (red)] (Fig. 2). Genotype B was detected in all samples devoted to W. Azerbaijan Province. In Kurdistan population, genotype B was also dominant and observed in four out of six specimens. From five individuals studied in Tehran Province, only one individual had a genotype B. Likewise, in Isfahan Province, genotype A was dominant and five out of six individuals had this sort of genotype. Nevertheless, in Kohgiluyeh-va-Boyerahmad Province, only genotype A was detected among the studied samples (Fig. 2). Therefore, two populations i.e., Azerbaijan and Kurdistan, were more similar to each other and three residues had more propinquity. The results of population genetics demonstrated that, 84% of all molecular diversity occurred in populations is related to within population diversity. However, 9% and 7% of remained variation were devoted to inter population and among regions diversity, respectively (Table 3, Fig. 3).

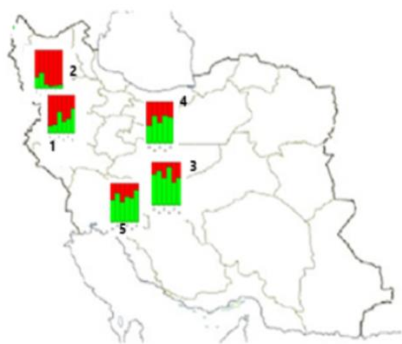

Fig. 2. Genetic diversity among the studied populations of *Salvia multicaulis* (1. Kurdistan, 2. Azerbaijan, 3. Isfahan, 4. Tehran, and 5. Kohgiluyeh-va-Boyerahmad Provinces) [Note: Genotype A and B are represented by green (lighter) and red (darker) colors, respectively].

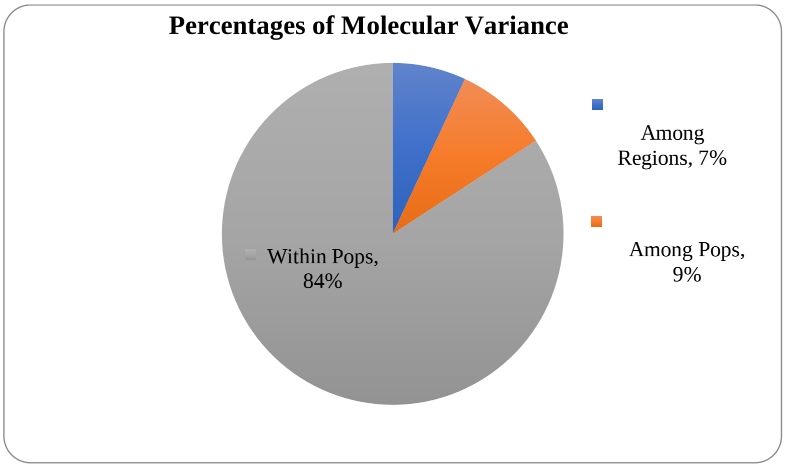

Fig. 3. The percentages of *Salvia multicaulis* molecular diversity: Between different regions (7%), inter population variation (9%), and intra population diversity (84%).

Table 3. Hierarchical AMOVA results for five populations of *Salvia multicaulis* (DF: Degree of Freedom, SS: Sum of Squares, VC: Variation Component, and PV: Percentage of Variation)

Source of variance	DF	SS	VC	PV
Among regions	2	110.157	1.943	7
Among populations	2	76.833	2.481	9
Within populations	24	564.733	23.531	84
Total	**28**	**751.724**	**27.955**	**100**

- Population genetic structure

The Evanno test and K-Means clustering produced the best number of genetic groups as K = 2. The STRUCTURE plot according to K = 2 is represented in figure 4. It showed close genetic affinity among five natural populations. Nevertheless, it also revealed that population 2, i.e., W. Azerbaijan, is differentiated in its genetic structure. Moreover, the plot demonstrated remarkable degree of genetic admixture among populations 3–5, afterwards population 1.

- Chemical compositions of the essential oil

The results of the essential oil compositions led to the recognition of 40 constituents extracted from five natural populations of *S. multicaulis*, ranging from 97.9% to 100% of the total chemical constituents**.** The results also demonstrated that α-pinene is among the three most abundant compounds in all the studied populations. However, the percentage of 1,8-cineole was higher than all other constituents in three natural populations i.e., Kohgiluyeh-va-Boyerahmad (20.4%), Tehran (19.0%), and Isfahan (17.6%), respectively. However, a considerable amount of this compound was also detected in two other populations (Table 4). Meanwhile, Camphor formed major component found only in two populations including Kurdistan (12.6%) and W. Azerbaijan (11.7%). Other compounds including E-caryophyllene [Kohgiluyeh-va-Boyerahmad (15.9%) and Kurdistan (12.4%)], Limonene [Tehran (14.1%)], and Bornyl acetate [Isfahan (13.0%)] constituted three most abundant compositions in the mentioned above populations. Nevertheless, a significant quantity of E-caryophyllene was observed in all the considered populations (Table 4).

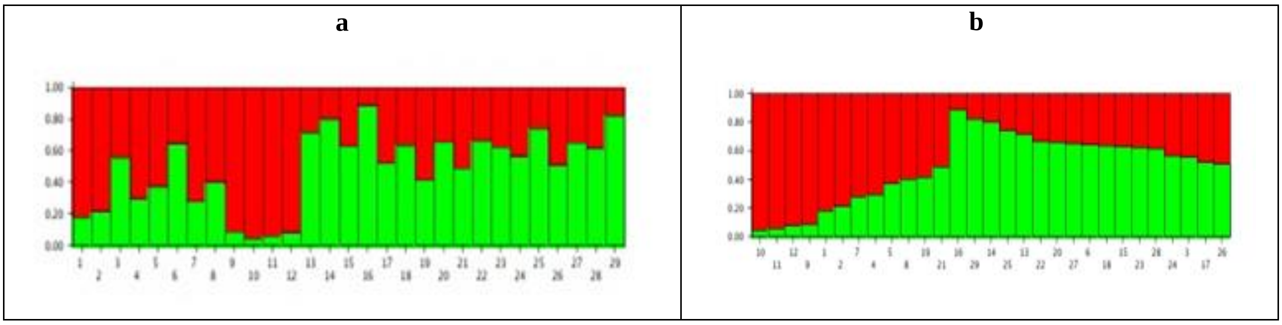

Fig. 4. The STRUCTURE plot of *Salvia multicaulis* populations: a. In order of placement in analysis, b. In order of Q amount (The individuals of populations 1–5 is based on table 1).

Table 4. The percentages of 40 essential oil components belonging to five natural populations of *Salvia multicaulis* (P1: Azerbaijan, P2: Kurdistan, P3: Tehran, P4: Isfahan, and P5: Kohgiluyeh-va-Boyerahmad). RI: Experimental Retention Indices, MS: Mass Spectroscopy, MI: Method of Identification, and RL: Literature Retention Indices (According to *Adams 2007, **Acree & Arn 2004)

Compound*	RI	RL	P1	P2	P3	P4	P5	MI
α–pinene	939	932*	11.2	12.4	16.5	17.1	19.9	RI, MS
Camphene	954	957**	6.6	4.0	6.0	7.0	7	RI, MS
β-pinene	979	974*	2.9	2.0	3.7	4.4	4.6	RI, MS
Myrcene	990	990**	1.8	1.0	1.9	2.6	2.5	RI, MS
O-cymene	1026	1022*	1.0	1.3	0	0	0	RI, MS
Limonene	1029	1022	3.4	3.4	14.1	6.6	8.4	RI, MS
1,8-cineole	1031	1026*	11.0	10.2	19.0	17.6	20.4	RI, MS
γ-terpinene	1059	1054*	0.4	0	0	0	0	RI, MS
Camphor	1146	1141*	11.7	12.6	4.0	6.3	3.7	RI, MS
Trans-pinocamphone	1162	-	0.8	0	0	0	0	RI, MS
Borneol	1169	1165*	3.4	4.5	4.9	8.5	5.8	RI, MS
Terpinen-4-o1	1177	1174*	1.5	3.1	1.1	0.7	1.1	RI, MS
Myrtenol	1195	1194*	3.9	2.6	1.2	0	0.9	RI, MS
Bornyl acetate	1288	1287*	4.6	5.5	5.3	13.0	6.6	RI, MS
Myrtenyl acetate	1326	1324*	1.3	1.1	0	0	0	RI, MS
α-copaene	1376	1374*	1.1	0	1.2	0	0	RI, MS
E-caryophyllene	1419	1417*	10.2	12.4	12.5	11.9	15.9	RI, MS
Z-β-farnesene	1442	1440*	0.7	0	0	0	0	RI, MS
α-humulene	1454	1452*	3.2	0.5	0	0	0	RI, MS
α-amorphene	1484	1483*	0.9	0	0	0	0	RI, MS
Bicyclogermacrene	1500	1500*	0.6	0	0	0	0	RI, MS
γ-cadinene	1513	1513*	0.8	0	0	0	0	RI, MS
δ-cadinene	1523	1522*	1.0	0	0	0	0	RI, MS
Caryophyllene oxide	1583	1582*	2.5	2.1	2.0	1.6	1.8	RI, MS
Diethyl phthalate (plastizer)	1590	1590*	2.5	0	0	0	0	RI, MS
10-epi-γ-eudesmol	1623	1622*	0.8	0	0	0	0	RI, MS
α-muurolol (=Torreyol)	1646	1644*	1.6	0	0	0	0	RI, MS
α-eudesmol	1653	1652*	2.5	0	0	0	0	RI, MS

Table 4 (contd)

epi-α-bisabolol	1684	1683*	2.4	0	0	0	0	RI, MS
14-hydroxy-(z)-4,5-dihydro caryophyllene	1706	1706*	1.7	0	0	0	0	RI, MS
1,4-cineole	978	1012*	0	2.2	0	0	0	RI, MS
2-methyl isoborneol	1181	1178*	0	6.3	0	0	0	RI, MS
Myrtenal	1195	1195*	0	0.4	0	0	0	RI, MS
α-cubebene	1348	1345*	0	0.4	0	0	0	RI, MS
9-epi-E-caryophyllene	1466	1464*	0	8.9	0	0	0	RI, MS
β-gurjunene	1433	1431*	0	0	1.5	0.7	0.7	RI, MS
p-cymene	1024	1020*	0	0	1.1	0	0	RI, MS
Valencene	1496	1496*	0	0	1.2	0	0	RI, MS
Aromadendrene	1441	1439*	0	0	0	0.6	0	RI, MS
Trans-cadina-1(6), 4-diene	1476	1475*	0	0	0	0.7	0	RI, MS
Total	**-**	**-**	**99.2**	**97.91**	**97.92**	**100**	**100**	**-**
The number of constituents in each population	-	-	30	21	17	15	14	-

The results of the cluster analysis based on Ward method is represented in figure 5. Two major groups were detected based on the essential oil compounds. The results indicated that, two populations encompassing Azerbaijan (No. 1) and Kurdistan (No. 2) were located in the taxonomic distance of seven and formed the first major group (Group A). Except for α-pinene and 1,8-cineole, that were in common in all populations, a remarkable amount of Camphor was only detected in two mentioned populations. The second major group (Group B) constituted the highest amounts of α-pinene, E-caryophyllene, and 1,8-cineole among all populations. PCA also confirmed this close relationship properly (Fig. 6).

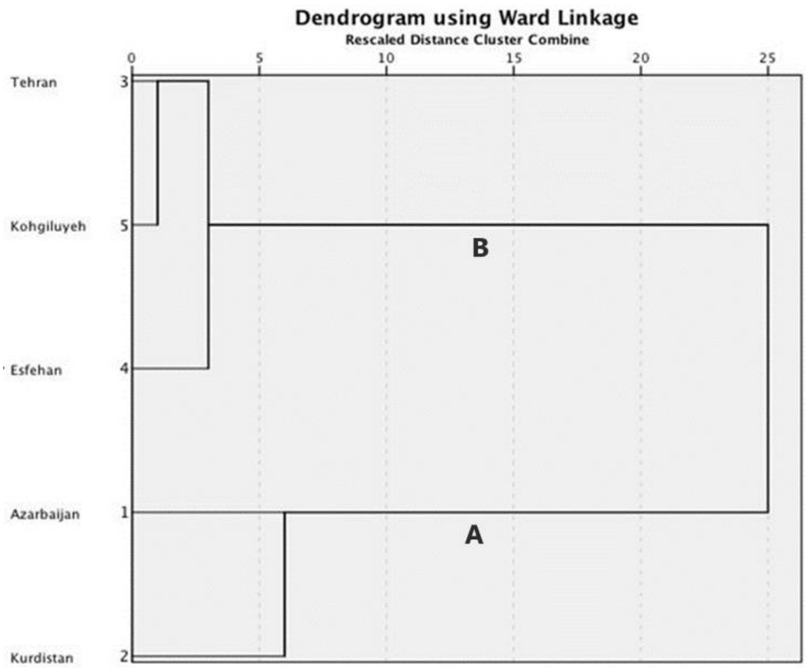

Fig. 5. The cluster dendrogram based on the essential oil compounds of *Salvia multicaulis* populations using ward method.

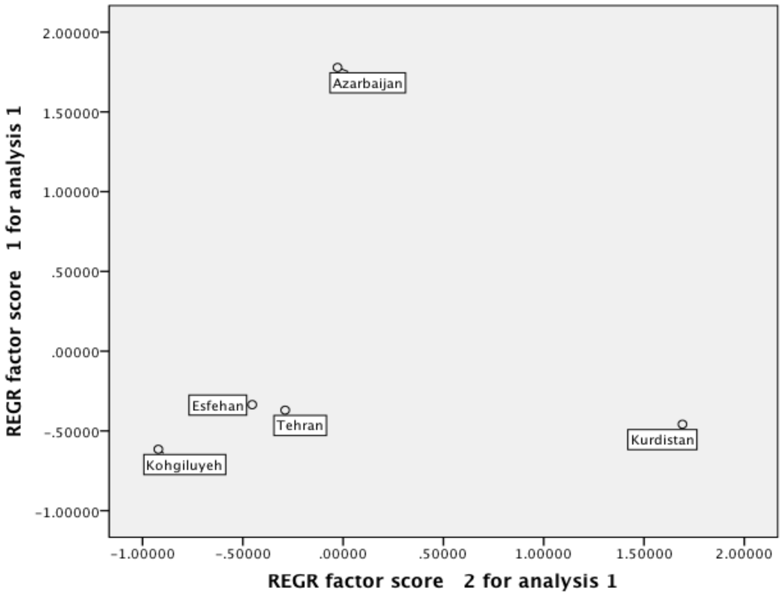

Fig. 6. Principle component analysis plot related to *Salvia multicaulis* populations based on essential oil compositions.

Discussion

The present study provided outstanding information about the genetic and essential oil composition variations of *Salvia multicaulis* populations in west, northwest, and center of the country. Considering genetic diversity is a necessary element for natural populations to evolve to adjust environmental changes (Frankham *et al.* 2002, Wang 2020), and regarding population genetics investigation could give rise to excellent data about the genetic structure and genetic variation of the populations (Sheidai *et al.* 2014), in this study, AFLP, as a DNA based molecular marker, was used in order to attain this goal. Recent study on genetic diversity of *S. multicaulis* populations was based on ISSR molecular markers (Talebi *et al.* 2021).

Gene flow and genetic differentiation play an important role in the evaluation of the population genetic structure. In other words, gene flow prevents genetic differentiation among populations (Hamrick & Godt 1990, Wang 2020). Therefore, the genetic drift lessens the within population genetic diversity (Setsuko *et al.* 2007, Sheidai *et al.* 2016). In the present study, the AMOVA test results demonstrated high amount of within population genetic diversity (84%) in *S. multicaulis* populations that could result in higher sustainability of populations against local environmental alterations (Table 3). The results of the STRUCTURE analysis showed some degree of genetic admixture among the studied populations. Therefore, limited gene flow was occurred among the populations. According to the latest study, the noteworthy molecular differences were observed among *S. multicaulis* populations using ISSR molecular markers (Talebi *et al.* 2021). Moreover, a considerable relationship was distinguished between the geographical distances and genetic variation, revealing a low gene flow quantity among the populations (Talebi *et al.* 2021). The reason might be due to the presence of substantial ecological obstacles such as Zagros mountains that affects gene flow (Li *et al.* 2012, Liu *et al.* 2013, Talebi *et al.* 2019, Talebi *et al.* 2021). Besides, high morphological diversity was previously distinguished among *S. multicaulis* populations, that confirms their genetic variations (Talebi *et al.* 2017).

The chemical results of the essential oil revealed significant differences among the studied populations in terms of various essential oil yields. This variation might be due to either environmental situations or genetics and physiological properties (Miguel *et al.* 2004, Talebi *et al.* 2019). Based on our results, α-pinene was determined among the three most abundant essential oil components in all-natural populations. The highest amount of this compound is reported from Kohgiluyeh-va-Boyerahmad population (19.9%). The result of this investigation was in accordance with some previous studies (Rustaiyan *et al.* 1999, Tepe *et al.* 2004, Fahed *et al.* 2016, Talebi *et al.* 2021). 1,8-cineole constituted the second major compound in all the studied populations. However, it was amongst the three most abundant components in four out of five populations including Kohgiluyeh-va-Boyerahmad, Tehran, Isfahan, and Azerbaijan. Talebi *et al.* (2021) also detected 1,8-cineole as the second major constituent. The third major content found in all populations was E-caryophyllene, that was amongst the three most plentiful components in Kohgiluyeh-va-Boyerahmad and Kurdistan populations. According to Rustaiyan *et al.* (1999) the major components were α-pinene (26.0%), 1,8-cineole+Limonene (20.0%) and Camphor (19.0%) that was similar to those obtained in this study. The highest amount of Camphor was only determined in Azerbaijan (11.7%) and Kurdistan (12.6%) populations. However, the major compounds attained from the aerial parts of a variety of this species in Lebanon were α-copaene (8.0%), α-pinene (6.6%), Myrtenol (5.7%), Trans-sabinyl acetate (5.3%) (Senatore *et al.* 2004). Nevertheless, in some populations mentioned by Talebi *et al.* (2021) the highest contents of Limonene have been determined. Surprisingly, in the present study, Limonene constituted the three most abundant compounds of Tehran population (Table 4). However, its contents in two other populations i.e., Isfahan and Kohgiluyeh-va-Boyerahmad was also remarkable (Table 4).

Limonene is regarded as an allelopathic agent found in arid environments (Erasto & Viljoen 2008, Talebi *et al*. 2021). It might have an adaptive role for plants (Talebi *et al.* 2021). Mohammadhoseini *et al.* (2008) determined Bornyl acetate (18.1%), E-caryophyllene (16.5%), and α-pinene (15.6%) as the main constituents of the flowering shoots. However, Bornyl acetate was also regarded as the three most abundant compounds after α-pinene in Isfahan population (13.0%). Morteza-Semnani *et al.* (2005) detected Camphor (11.0%), 1,8-cineole (10.7%), Borneol (8.6%), and α-pinene (7.5%) as the major components from the north of the country. Overall, α-pinene, 1,8-cineole, and E-caryophyllene were regarded as the main components found in considerable amount in all the studied populations. However, other major constituents including Camphor, Limonene, and Bornyl acetate were not in acceptable amounts in all local populations.

There are a few investigations about the environmental factor effects on the essential oil composition of *Salvia multicaulis* species. However, the recent study on this species, demonstrated that some ecological factors including climate changes, altitude, and soil salinity, could affect the essential oil amounts (Tavakoli *et al.* 2022). Although most of the chemical components of *S. multicaulis* were similar in most of the studied regions, the compounds percentages were miscellaneous. These results were in accordance with those obtained in this study. Moreover, they found altitude as the most important factor contributed to the essential oil contents as well as oxygenated terpenes.

Generally, higher altitudes can give rise to higher environmental tensions for plant and such elements have effective role on the essential oil contents. Commonly, stressful environment has a crucial role in synthesizing more antioxidant compounds. The antioxidant properties of the essential oil are mostly related to the oxygenated terpene contents which have a positive relationship with altitude (Amorati *et al*. 2013). The results of the present study, affirmed this claim to some extent (Table 4). However, based on Fernández-Sestelo *et al.* (2020) there is a noteworthy negative relationship between altitude and 1,8-cineole and Camphor (as monoterpenes) proportions.

Climate change and soil parameters are considered other environmental factors that affect chemical compounds. Sefidkon *et al*. (2005) reported that, climate changes are the most important environmental factors that are effective on 1,8-cineole compound percentage. The results of this study also conform with those obtained by previous investigations. Based on Tavakoli *et al.* (2022), climate changes, including precipitation amount and relative humidity, are regarded as the most significant parameters efficient in monoterpenoid and sesquiterpenoid percentages.

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